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W. C. Mckay

November 1983

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of Fisheries and Aquatic
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et aquatiques nº 1163

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Compte-rendu des communications du neuvième atelier annuel sur la toxicité aquatique : du 1 an 5 novembre 1982, Edmonton (Alberta)

Editor/Éditeur

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SYNOPSIS OF THE NINTH ANNUAL AQUATIC TOXICITY WORKSHOP

Aquatic Toxicity Workshops have been held annually since 1974 when the first session was hosted by the Freshwater Institute in Winnipeg. The Workshop has provided a forum for discussion and information exchange in the disciplines composing the field of aquatic toxicology. It has been the intent of the National Steering Committee and the individual Workshop Committees, at the request of the delegates, to keep the Workshop atmosphere informal. Provision of ample opportunities to discuss relevant issues in aquatic toxicology with colleagues from government agencies, industry, consultants and universities has been as important to the organizers as the sessions themselves. These objectives were clearly accomplished at the Ninth Annual Workshop.

The Ninth Annual Aquatic Toxicity Workshop was held from November 1-5, 1982 at the Four Seasons Hotel in Edmonton. The Workshop featured the following themes:

1) the role of aquatic toxicological research in the development of energy, resources and other large-scale industrial and municipal projects.

2) the advancement of aquatic toxicology as a scientific discipline.

3) the development and use of risk assessment and evaluation methods in setting criteria to assure the protection of the environment.

The Workshop was divided into two non-concurrent sessions, eleven concurrent sessions and three workshop sessions. The non-concurrent and concurrent sessions were divided into formal papers (20 minutes) and informal papers (10 minutes). A total of thirty-two papers were presented.

RÉSUMÉ DU NEUVIÈME ATELIER ANNUEL SUR LA TOXICITÉ EN MILIEU AQUATIQUE

Les ateliers sur la toxicité en milieu aquatique ont lieu chaque année depuis 1974, date à laquelle la première séance a été organisée par le Freshwater Institute de Winnipeg. Les ateliers constituent un forum pour la discussion et l'échange de données dans les disciplines qui composent l'étude de la toxicité en milieu aquatique. À la demande des délégués, le Comité directeur national et les différents comités d'ateliers se sont toujours efforcés de faire en sorte que l'ambiance des ateliers soit décontractée. Les organisateurs ont toujours accordé une importance égale aux séances et aux possibilités de discuter avec des collègues venant du gouvernement, de l'industrie, des agences de consultation ou des universités. Le neuvième atelier a bien su allier les deux objectifs que s'étaient fixés les organisateurs.

L'atelier s'est tenu du 1 au 5 novembre 1982 à l'hôtel Quatre-Saisons d' Edmonton. On y a abordé les thèmes suivants:

1) Le rôle de la recherche sur la toxicité en milieu aquatique vis-à-vis du développement des ressources énergétiques et des autres projets industriels et municipaux à grande échelle.

2) l'avancement de la toxicologie en milieu aquatique, comme discipline scientifique.

3) la mise au point et l'utilisation de méthodes d'évaluation et d'estimation des risques permettant de postuler des critères qui pourraient assurer la protection de l' environnement.

L'atelier s'est subdivisé en deux séances non parallèles, onze séances parallèles et trois séances d'atelier. Les séances parallèles et non parallèles étaient consacrées à des conférences (d'une durée de 20 minutes) ou à des présentations (10 minutes). On a fait, au total, trente-deux exposés.

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ACKNOWLEDGMENTS:

On behalf of the Ninth Annual Aquatic Toxicity Workshop Organizing Committee, I would like to take this opportunity to acknowledge the contributions and support of a number of people and organizations whose support made this workshop a success.

The gracious hospitality of Larry Cooper and the staff of the Four Seasons Hotel made the stay of all participants enjoyable.

The design for the workshop logo and of the brochures advertising the workshop were the responsibility of Tom Tinkler and his staff in the Creative Services, Communications Production Group, Public Affairs Bureau, Alberta Government.

The Organizing Committee would also like to express its gratitude to the following agencies for their support: Alberta Environmental Centre, Research Management Division, Water Quality Control Branch, Environmental Protection Service, AOSTRA, Syncrude Canada, Suncor, Hospitality and Promotions Branch, The Kananaskis Centre for Environmental Research and the University of Alberta.

We also wish to thank our two guest speakers, the participants in the workshop sessions and the session chairpersons.

GUEST SPEAKERS

Dr. E.E. Ballantyne
Dr. S. Hrudey

WORKSHOP PARTICIPANTS

M. Speyer
M. Taylor
G. Craig
R. Wilson

M. Prior
L. Lillie

L. Duncan
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B. Munson
M. Strosher

We would like to thank P. Durocher and R. Gauthier of Fisheries and Oceans, Environment Canada for the Seminar on WATDOC.

On behalf of the editorial committee, I would like to express our appreciation to Jim Bradley and Diana Lee from the Alberta Environmental Centre for their assistance in preparing the proceedings and the people of the Translations Service, Alberta Culture who provided the necessary translators.

One person who does not appear on the list of members of the Organizing Committee but who did so much for us is our secretary Ramona Ergezinger.

Lastly we would like to acknowledge the workshop delegates. The enthusiasm and comradery you displayed made this workshop a memorable occasion for us all.

W. H. Lake
Workshop Chairman
January 1983.

COMITÉ ORGANISATEUR DE L'ATELIER

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	M. Strosher	Université de Calgary
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Alberta Oil Sands Technology and Research

Environnement Canada

Service de protection de l'environnement

Suncor

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Au nom du comité organisateur du neuvième atelier annuel sur la toxicité en milieu aquatique, j'aimerais remercier les personnes et les organismes qui, par leur travail ou leur appui,ont contribué en large mesure à son succès.

L'accueil chaleureux de Larry Cooper et du personnel de l'hôtel a rendu plus agréable le séjour de tous les participants.

La conception du sigle de ateliers et des brochures connexes révient à Tom Tinkler et à son personnel des Services de création du Groupe de production des communications, Bureau des relations publiques, gouvernement de l'Alberta.

Le Comité organisateur tient également à remercier les organismes suivants de leur appui: le Centre de l'environnement de l'Alberta, la Direction de l'administration de la recherche, la Division du contrôle de la qualité des eaux, AOSTRA (le Bureau de recherche et de technologie des sables bitumineux de l'Alberta), Syncrude Canada, Suncor, la Division de l'accueil et de la promotion, le Centre Kananaskis de recherche sur l'environnement et l'Université de l'Alberta.

Nou tenons par ailleurs à remercier nos deux orateurs invités, les participants aux ateliers et les présidents de séance.

Orateurs invités

E.E. Ballantyne
S. Hrudey

Membres des ateliers

M. Speyer
M. Taylor
G. Craig
R. Wilson

M. Prior
L. Lillie

L. Duncan
J. Flett
W. Smart

Présidents de séance

G. Craig
S. Leonhard
W. Mackay
H. Boerger
Y. Hoyano
B. MacDonald
B. Hammond
J. Kostler
A. Beckett
S. Ramamoorthy
B. Munson
M. Strosher

Nous remercions de plus MM. P. Durocher et R. Gauthier d'Environnement Canada, de leur colloque sur le système WATDOC. (Méthode de documentation concernant les systèmes de données sur les ressources en eau).

Au nom du Comité de rédaction, je veux exprimer nos sincères remerciements à Jim Bradley et Diana Lee du Centre de l'environnement de l'Alberta de leur aide, ainsi qu'au Service de traduction du gouvernement de l'Alberta, qui nous a rendu un fier service.

Par ailleurs, il s'impose de remercier une personne qui nous a beaucoup aidés, mais dont le nom m'apparaît pas sur la liste des membres du Comité organisateur, à savoir, R. Ergezinger, notre secrétaire.

Enfin, nous tenons à remercier tous les délégués. Votre enthousiasme et l'esprit de camaraderie que vous avez manifestés ont fait de notre colloque une occasion inoubliable.

Le Président de l'Atelier,

W. H. Lake

janvier 1983.

EDITORS'S COMMENTS

The Ninth Annual Aquatic Toxicity Workshop has achieved international status. These Proceedings include papers from five countries which deal with topics that range from basic research through analysis of regulatory policies concerning the quality of the aquatic environment.

The papers included here report on:work done in both freshwater and marine environments, the chemistry of a range of toxicants under various conditions, and the effects of a variety of toxicants on a wide range of both plant and animal. A common thread linking all papers is their relevance to practical problems being experienced in maintaining the quality of aquatic environments. They represent a cross section of the kind of work presently being done in aquatic toxicology.

Eighteen manuscripts were presented for publication in these Proceedings. All were reviewed by at least two referees. One was accepted as submitted and the remainder required major or minor revisions.

Program abstracts and extended abstracts are reproduced as received, except for minor editorial alterations. Abstracts of two papers (Chang *et al*. and Garie *et al*.) are included although the authors were unable to attend the workshop at the last minute.

The text of the talk of one of the workshop participants, M. Prior, is included, because of the perspective he presents for aquatic toxicologists.

W. C. Mackay

A SIMPLE MICROASSAY TECHNIQUE FOR MEASURING ALGAL GROWTH INHIBITION (EC50's) IN AQUATIC TOXICITY STUDIES

Christian Blaise, Richard Legault and Norman Bermingham

Environmental Protection Service, Environment Canada
Longueuil, Quebec

BLAISE, Christian, Richard LEGAULT and Norman BERMINGHAM. 1982. A simple microassay technique for measuring algal growth inhitition (EC50's) in aquatic toxicity studies. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

A miniaturization of the conventional EC50 algal assay procedure utilized for aquatic toxicity determinations was undertaken. This technique consists in injecting individual wells of rigid disposable styrene microplates with 10µL of algal inoculum (Selenastrum capricornutum Printz), 10µL of a synthetic medium growth spike and 200 µL of the appropriate serial dilution of the test sample. Comparison of the microassay and conventional techniques following toxic evaluation of several industrial effluents suggests that the microplate procedure could advantageously be substituted for the conventional test in conducting basic algal bioassays. Other interesting features of the microassay technique include handling rapidity, economy, space-saving convenience, and automation potential.

Key Words: Growth inhibition, Selenastrum capricornutum, freshwater algae.

BLAISE, Christian, Richard LEGAULT and Norman BERMINGHAM. 1982. Mesure de l'inhibition de la croissance algale (CI$_{50}$) pour les etudes de toxicite aquatique a l'aide d'une microtechnique simple. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Nous avons entrepris de miniaturiser la procédure conventionelle de bioanalyse utilisée pour la mesure de la CI$_{50}$ avec algues afin d'évaluer la toxicité aquatique. Par cette technique, on introduit, dans chaque puits d'une microplaque faite de plastique en styrène rigide (jetable), les volumes suivants: 10µL d'une suspension d'algues de Selenastrum capricornutum Printz; 10 µL d'un ajout de milieu de croissance synthétique; 200 µL de la dilution en série appropriée de l'échantillon à l'etude. Suite à la comparaison des deux techniques (conventionnelle et avec microplaque) ou l'évaluation toxique de plusieurs effluents industriels fut effectuée, il apparaît probable que la procédure avec microplaque pourrait avantageusement remplacer le test conventionnel pour la réalisation de bioessais routiniers avec algues. En plus, cette microtechnique se distingue davantage par sa rapidité d'exécution, son coût, son économie d'espace, et son potentiel d'automatisation.

INTRODUCTION

The recognition of algae as biological indicators of environmental quality is today a *fait accompli* and their various modes of application for assessment of aquatic pollutants are well documented (Couture, 1981; Rai and colleagues, 1981; USEPA, 1978). For toxicological and more recently ecotoxicological evaluations of effluents, solid waste leachates, and other complex mixtures, our laboratory routinely determines 8-day EC50's utilizing the green alga Selenastrum capricornutum Printz with a toxicity test procedure closely resembling that of Keighan (1977) and incubation conditions quite similar to those of USEPA (1978). Such widely used conventional testing methods using flasks as culture vessels, although basically practical and reliable, are not without disadvantages. Filtering of samples across 0.45u membranes when cell densities are to be measured using electronic particle counters, for instance, can be time consuming, particularly if suspended materials are present in the sample. Also, test preparatory manipulations such as dilution preparation and the pipetting of macrovolumes (25 mL or more) into individual flasks can be more or less laborious depending on laboratory set-up and practices. Incubator space requirements can be another potential disadvantage of using flasks. Again the specific post-experimental washing procedure which must be applied to flasks reused for algal bioassays is critical and therefore usually performed by the testers themselves, resulting in a further imposition of time and effort. Finally, continuous reuse of glass testing vessels can bring about changes in the optical properties of certain flasks (due to aging and scratching, for example), as well as contamination and toxicity problems - all of which can lead to data variability. Furthermore, in service laboratories such as ours where limited personnel are required to undertake multiple indicator bioassays on a regular basis, any time-consuming operation(s) will ultimately have a negative impact on overall productivity output. Being able to perform algal assays, therefore, by means of a more easily-manageable test without the above inconveniences became a desirable objective.

The idea that Microtiter^R plates, used extensively in the field of clinical microbiology and for which applications are numerous (Handbook of Microtiter Procedures, 1972), might successfully be exploited to develop a simple miniaturized algal toxicity test was thus investigated. Environmental applications utilizing microplates are not extensive. Jennett and colleagues (1977), however, have developed a quantitative bioaccumulation screening method for unicellular and filamentous green and blue-green algae with microplates. More recently, Dive (1981) has utilized similar plates and successfully developed a miniaturized viability test for ecotoxicity evaluations with Colpidium campylum, a ciliated protozoan. Our recent efforts to develop a microplate toxicity assay with the green alga S. capricornutum and results obtained thus far are the subject of this paper.

MATERIALS AND METHODS

Several types of microplates are available commercially, basically differing in terms of plastic composition and well shape. For our initial experiments, we have arbitrarily chosen to work with rigid polystyrene plastic plates with U-shaped wells. The microassay technique developed thus far is as follows (Figure 1):

1. Serial dilutions of the sample are prepared in test tubes (filtered sample volume requirement is 10 mL).
2. With an Eppendorf repeating pipette, 200 uL of Millipore (Super Q water system) control water are added to each of the eight wells of column 12 in each microplate that is to be used for testing a specific water sample; 200 uL of the highest sample dilution and succeeding smaller dilutions are then respectively added to each well of columns 11, 10, 9, and so on, down to column 1.
3. A synthetic nutrient spike of AAP medium (USEPA, 1978) is prepared and added to an appropriate dilution of algal cells such that dispensing 20 uL of this mixture (again, with an Eppendorf repeating pipette) in each well of the microplate will correspond to a theoretical algal growth yield of 50 ppm and initial algal inoculum of 1000 cells/mL respectively.
4. Plate cover is put on top of the microplate which is then placed in a transparent plastic bag (heat-sealable pouches, KAPAK Corporation) whose open end is then heat sealed to prevent evaporation.
5. Each plate is then incubated for 8 days under conditions identical to those of the conventional test.
6. Microplates are stirred for one minute twice daily with a microplate stirrer (Fisher Scientific Co. Minimix).
7. At day 8 (end of incubation period), microplates are stirred thoroughly with the microstirrer to resuspend algal cells: 100 uL aliquots are then removed from each well with a micropipette and placed into an accuvette containing 9 mL of isoton electrolyte solution. Cell counts are determined with a model TA II Coulter Counter instrument with subsequent transformation

to biomass (ppm) by means of a conversion formula relating day-8 counts in control wells with the theoretical algal biomass yield of the synthetic medium spike (50 ppm in our case).

RESULTS

An initial investigation with the microplate system was aimed at verifying sample volume loss due to evaporation under experimental conditions of sealing as described previously (see Materials and Methods section and Figure 1). A volume of 220 uL of Millipore Super Q water was micropipetted into each of the 96 wells of a microplate which was then sealed and incubated for 8 days. The volume remaining in one out of every two of the 96 wells of the microplate was then removed by micropipette once more and determined by weighing on a sensitive digital self-zeroing balance (Sartorius Model 3705).

Concurrently, 24 conventional 125 mL test flasks each containing 40 mL of Millipore Super Q water were placed in the same incubator to estimate sample volume loss after 8 days. Results indicated that the mean sample volume loss for both methods was under 10% and that differences between the two methods in this respect were not significant (Table 1). The microplate sealing technique described was thus employed for future tests.

After having undertaken ensuing algal bioassays of several industrial effluent samples to compare conventional and microplate techniques, sufficient data became available to estimate variability of the microassay technique. As results show in Table 2, test variability is well under the 15% allowable variability index recognized by USEPA for conventional algal bioassays (USEPA, 1971). These results also indicate that the micropipetting instruments and technique utilized to dispense microvolumes are adequate and precise enough to ensure reproducibility of data.

Although not yet fully validated, preliminary investigations concerning various aspects of the microplate technique have indicated the following:

1. Algal cell inocula of 5,000, 2,500, or 1,000/mL do not significantly change the total count obtained after 8 days of incubation. We have therefore kept the 1000/mL algal cell inoculum used for the conventional flask test.
2. The sample volume to experimental vessel volume ratio used for the microplate procedure described (i.e., 220 uL sample volume in a 300 uL capacity well) is one of 0.73, whereas USEPA (1978) recommends that such a ratio should not exceed 0.20 to avoid CO_2 limitation. Recent control growth tests performed with ratios of 0.73, 0.50, 0.40, and 0.27, however, have not shown significant differences in cell counts/mL obtained after 8 days (Table 3). One possible explanation is that the plastic cover placed over the microplate is not air tight. This means that the experimental vessel volume is theoretically that volume contained within the sealed plastic bag covering the microplate and its cover, in which case the above ratio would be well below the 0.20 value advocated by USEPA (1978).
3. Maximum standing crop for controls obtained after eight days of microplate and flask incubation, as reflected in cell counts/mL, is not significantly different. This would seem to indicate that the quality and intensity of light reaching algal cells in the microplate wells for photosynthesis is similar to that available to algal cells in glass flasks. It appears, therefore, that the microplate sealing technique employed to control evaporation is not a factor inhibiting algal growth. This assumption will be further verified as comparative data between the microplate and flask techniques accumulate.

Very recent comparative microplate and flask EC50 toxicity data have been obtained with seven (7) industrial effluent samples. These are shown in Table 4. Only one of the samples tested displayed a dissimilar EC50 concentration range after being assayed by both methods. This is not particularly unusual, we feel, and can be explained by the fact that the toxicity data reported here result from cell densities obtained with an electronic particle counter. Although effluent samples subjected to our algal assays are 0.45u membrane-filtered prior to testing, such a treatment will not ensure bacteria-sterile conditions in the ensuing filtered samples. In fact, no particular effort is made in attempting to maintain sample sterility since we do not utilize an axenic culture of S. capricornutum for inoculating purposes. Hence, bacterial growth is likely to occur during the 8-day bioassay period. Such growth is indeed a frequent occurrence in pulp and paper effluent samples because of the presence of nutrients favorable for microbial growth. Significant bacterial activity was confirmed for sample 5 by microscopical observations in both microplate and flask concentrations lying in the vicinity of the EC50 value range. Various clumpings of bacteria and algae were observed. This would likely favor Coulter cell count variability and probably explain the incompatible comparative data obtained for sample 5. Similar shortcomings and others have been reported for algal bioassays making use of electronic particle counters (Rehnberg and colleagues, 1982). This explains, to some extent, our preference in comparing microplate and flask procedure data by presenting EC50 concentration ranges instead of calculated EC50 values. Other reasons why reporting the range of EC50 values is preferable to reporting absolute EC50 values have been given by Van Coillie and colleagues (1982) and by Couture and colleagues (1982).

Lastly, an interesting comparative estimate has been made between the microplate and flask assay techniques with regards to manipulative rapidity of sample preparation for incubation (Table 5). Based on our current laboratory set-up and practice for initiating flask bioassays, it appears that one sample could be processed approximately 3 times faster using the microplate technique.

DISCUSSION

Much additional investigative work still remains to be performed with the microassay technique before such a procedure can achieve validation for routine algal EC50 determinations in aquatic toxicity studies. For one thing, considerable data generation comparing this miniaturized microplate technique with the conventional flask bioassay will be required. Again, work of a more fundamental nature to be undertaken in future method development will attempt to yield information concerning the following:

1. selection of the most suitable microplate plastic type (polystyrene, polyvinyl, etc.), brand, and shape of wells;
2. selection of the most suitable sample volume to test vessel volume ratio;
3. selection of the most efficient microplate sealing technique;
4. selection of the most appropriate incubation period (1, 4 or 8 days) for EC50 determinations;
5. selection of a choice parameter in reporting toxicity effects (electronic particle counter, ATP, Δ- fluorescence, etc. ?).

While such questions remain to be answered, preliminary development and test results presented at this time are encouraging and appear to indicate interesting possibilities for the microplate technique. Apart from its simple, rapid, economic, and space-saving features, this technique, if coupled with recent instrumentation technology designed around microplates offers the added potential of virtually complete automation. Based on the limited knowledge available at this time, we nevertheless feel that this technique could now be used successfully for screening purposes and as a preliminary test preceding a final assay using a conventional flask procedure.

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Figure 1; Microplate algal assay technique.

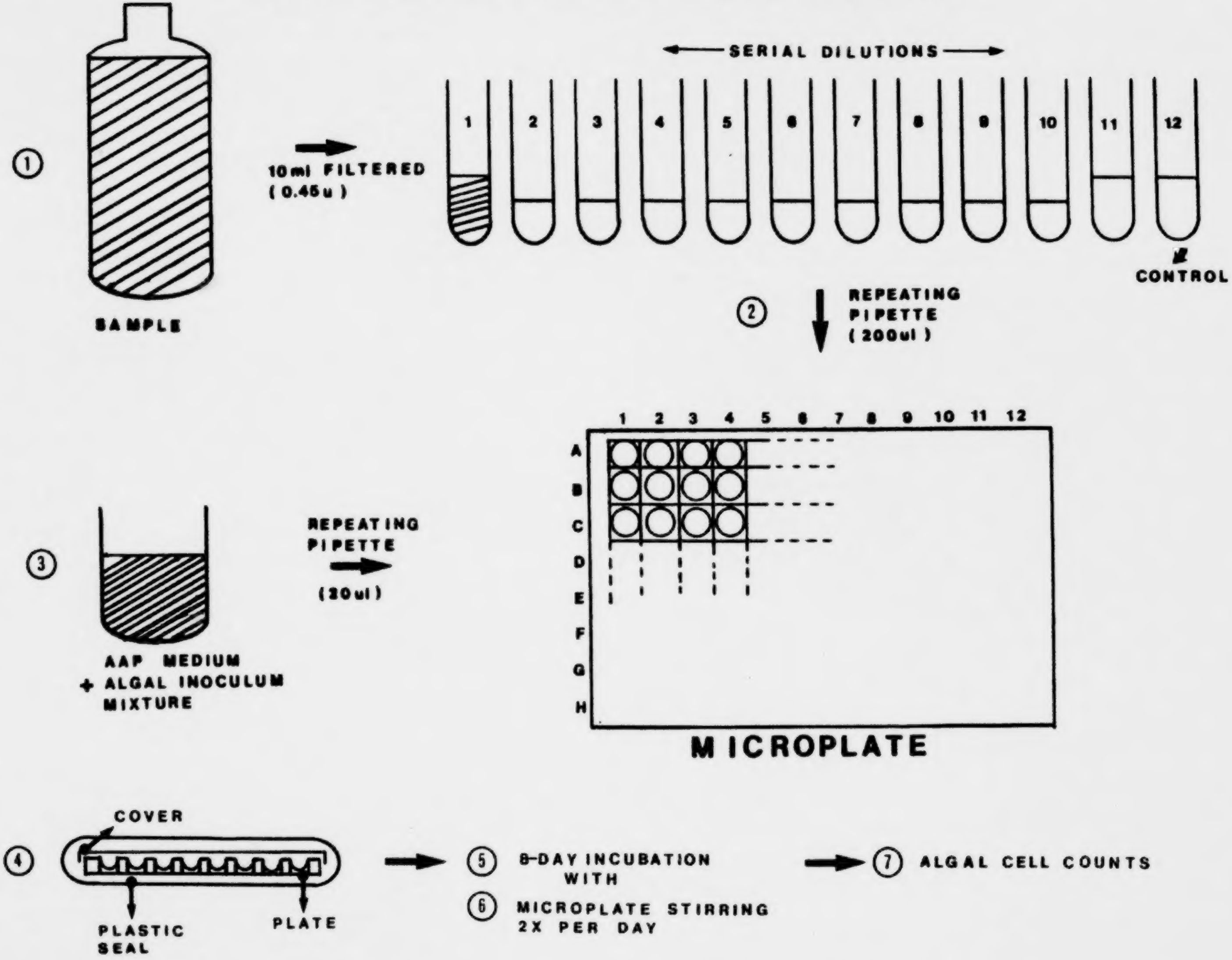
①
SAMPLE
10ml FILTERED
(0.45u)
SERIAL DILUTIONS
1
2
3
4
5
6
7
8
9
10
11
12
CONTROL
②
REPEATING
PIPETTE
(200ul)
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
MICROPLATE
③
AAP MEDIUM
+ ALGAL INOCULUM
MIXTURE
REPEATING
PIPETTE
(20ul)
④
COVER
PLASTIC
SEAL
PLATE
⑤ 8-DAY INCUBATION
WITH
⑥ MICROPLATE STIRRING
2X PER DAY
⑦ ALGAL CELL COUNTS

TABLE 1: SAMPLE VOLUME LOSS DUE TO EVAPORATION AFTER 8 - DAY INCUBATION PERIOD FOR CONVENTIONAL FLASK ASSAY METHOD (CFAM) AND MICROPLATE TECHNIQUE (MPT).

ASSAY METHOD	n (FLASKS/WELLS)	MEAN Δ % VOLUME LOSS	STANDARD DEVIATION
CFAM*	24	7.2	± 2.8
MPT**	48	8.7	± 2.7

* 40 mL sample volume in 125 mL flask

** 220 uL sample volume in a 300 uL capacity well.

TABLE 2: VARIABILITY OF THE ALGAL MICROASSAY TECHNIQUE AS INDICATED BY COEFFICIENTS OF VARIATION (C.V.) CALCULATED FROM CONTROL WELLS OF 18 MICROPLATES

C.V.* VALUES OBTAINED	MEAN C.V.
4.5, 5.4, 6.0, 6.2, 6.5, 6.9, 7.0, 7.2, 7.2, 7.3, 7.6, 8.1, 8.4, 9.3, 9.8, 11.3, 11.6, 13.9	8.0

* Calculated from modified Coulter Counter cell numbers obtained at end of incubation period (Day 8) (n = 8 for each C.V. value reported).

$$\text{C.V.} = \frac{(\text{Standard Deviation}) \times 100}{\bar{X}}$$

TABLE 3: SAMPLE VOLUME TO EXPERIMENTAL VESSEL VOLUME RATIOS (S/EV) FOR MICROPLATE (M) AND FLASK (F) PROCEDURES

PROCEDURE	SAMPLE VOLUME	VESSEL VOLUME	S/EV	
M	220 uL	300 uL	0.73	8 - DAY CELL COUNT PER mL NOT SIGNIFICANTLY DIFFERENT ACCORDING TO RECENT TESTS
M	150 uL	300 uL	0.50	
M	120 uL	300 uL	0.40	
M	80 uL	300 uL	0.27	
F (OUR LAB)	40 mL	125 mL	0.32	
F (EPA)	25 mL	125 mL	0.20	

TABLE 4: COMPARATIVE MICROPLATE (MP) AND FLASK (F) EC50 TOXICITY DATA OBTAINED WITH 7 INDUSTRIAL EFFLUENT SAMPLES

SAMPLE	EC50 CONCENTRATION RANGE	
	MP	F
1 (CPE)	0.050 - 0.10	0.06 - 0.13
2 (PPE)	0.39 - 0.78	0.39 - 0.78
3 (PPE)	0.78 - 1.56	0.78 - 1.56
4 (PPE)	1.56 - 3.13	1.56 - 3.13
5 (PPE)	6.25 - 12.5	3.13 - 6.25
6 (PPE)	12.5 - 25	12.5 - 25
7 (PPE)	12.5 - 25	12.5 - 25

CPE: CHEMICAL PIGMENT EFFLUENT
PPE: PULP AND PAPER EFFLUENT

TABLE 5: RELATIVE TIME SPENT IN ALGAL BIOASSAY PREPARATION FOR THE CONVENTIONAL FLASK TEST (CFT) AND THE MICROPLATE TEST (MP)

	TIME IN MINUTES	
OPERATION	CFT	MP
SERIAL DILUTION PREPARATION AND DISPENSING *	40	16
ADDITION OF NUTRITIVE SPIKE AND ALGAL INOCULUM	10	2
TOTAL TIME	50	18

* Based on the preparation of 11 different effluent concentrations and one control; in replicates of 5 for CFT and of 8 for MP.

CADMIUM COMPLEXATION BY HUMIC SUBSTANCES : CHEMICAL AND ECOTOXICOLOGICAL STUDY WITH CILIATE PROTOZOAN COLPIDIUM CAMPYLUM

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DIVE, D., N. POMMERY, M. LALANDE and F.ERB. 1982. Cadmium complexation by humic substances : chemical and ecotoxicological study with ciliate protozoan Colpidium Campylum. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The complexation of low concentrations of cadmium (0 to 60 µg.L^{-1}) in the presence of two different humic substances was studied in three different media; the toxicity of mixtures for Colpidium Campylum (ciliate protozoan) was assessed by a factorial experimental design. Results show that the chemical composition of the medium strikingly influences the complexation of the metal and the ecotoxicity. The growth inhibition in each medium differs more in relation to the medium components than in relation to free cadmium concentration even if the presence of humic substances has an antagonistic action on the metal toxicity. A medium has been selected to compare commercial and natural humic substances. It appears that in this medium, complexation abilities are different for the two types of humic substances and the response of Colpidium depends essentially on free cadmium concentration in the medium.

Key Words: Cadmium, humic substances, protozoan, Colpidium Campylum, complexation, growth inhibition.

DIVE, D., N. POMMERY, M. LALANDE and F. ERB. 1982. Complexation du cadmium par les substances humiques : etude chimique et ecotoxicologique a l'aide du protozoaire cilie Colpidium Campylum. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

La complexation de concentrations faibles de cadmium (0 à 60 µg.L^{-1}) en présence de deux types différents de substances humiques a été étudiée et la toxicite des mélanges pour Colpidium Campylum (Protozoaire Cilié) mesurée par des expériences factorielles. Les résultats montrent que la composition chimique du milieu exerce une influence nette sur la complexation et la toxicité du métal. L'inhibition de croissance observée dans chacun des milieux dépend plus de la nature du milieu que de la concentration de cadmium libre, même si la présence de substances humiques exerce un effet détoxifiant. Un milieu a été sélectionné pour comparer les substances humiques commerciales et naturelles. Il apparait que, dans un milieu donné, la complexation est différente selon l'origine des substances humiques et que la réponse de Colpidium dépend alors essentiellement de la concentration de cadmium libre présente dans le milieu.

INTRODUCTION

Numerous studies have demonstrated the complexation of metals by natural ligands present in water (1-5). Several investigators have studied the modification of toxicity and bioaccumulation of metals in organs by complexing agents such as EDTA, NTA, and DPTA (6, 7, 8)

As concerns humic material, the amounts of complexed metals are so limited in natural conditions that very sensitive methods are needed to detect the difference in toxicity caused by the fraction of metal bound to humic material. The observation of sublethal effects on *Daphnia Magna* during 21 to 30 days is a good, but very long method and the detection threshold of short-term methods is rarely sufficient for such studies.

Colpidium Campylum can be used as a model for the evaluation of mineral and organic pollutants (9).

The purpose of the present study was to evaluate *Colpidium Campylum* as a Ciliate Protozoan model for estimation of the effects of the complexing action of humic material on cadmium toxicity.

MATERIAL AND METHODS

Humic Material

Humic materials used were commercial (CH) and natural (NH).

Commercial humic material (FLUKA AG Buchs SG, Switzerland) (85 to 90% purity) have a substantial complexing effect on metals. They are composed of 80% high molecular weight structures (MW >300.000) which are well known to form very stable complexes with metals (1). The lightest fractions (MW <5,000) are proportionally able to complex much more metal than the heaviest ones, but complexes formed in the presence of low molecular weight fraction are less stable (1).

Natural humic materials were extracted from pond water (10) using Amberlite XAD 8 adsorbant resin. The composition of the NH material used during this study, which was very similar to fulvic acids, is:

	MW	50,000	none
50,000	MW	5,000	30 to 38%
5,000	MW	500	50 to 54%
	MW	500	6 to 8%

Preparation of Samples and Measurement of Free Cadmium Levels

Addition of cadmium to solutions of humic material was performed from a 1 g·l^{-1} solution of Cd $(NO_3)_2$, three days before the experiment, to reach the equilibrium between free and complexed cadmium species (Erb et al. unpublished results).

Free cadmium was measured, using differential pulse anodic stripping voltammetry (DPASV) with an EG & G PARC polarograph (model 174 A).

Protozoan Test

Toxicity of cadmium for *Colpidium Campylum* was performed, using the method previously described by Dive and Leclerc (II) and standardized according to the following protocol:

flasks: plastic cylindrical screw caped flasks	30 mL
cadmium - humic material sample in medium concentration 1.25 X	4 mL
48 h culture of *C. Campylum* on *E. Coli*	s.a.f.(*) 5,000 cells
Dry *E. Coli* (EC sigma) in medium (5 mg·mL^{-1})	0.2 mL
Medium	s.a.f. (*) 5 mL

Each mixture was tested in triplicate. Populations in flasks (N) were enumerated after 24 h incubation at 28°C with a Coulter Counter (200 µm aperture) and the number of generations (NG) calculated by the relation:

$$NG = \log_{(2)}\left(\frac{N}{1000}\right)$$

Three different media were used for the study. They were selected on the basis of their ability to promote monoxenic growth of Ciliates.

Their composition (similar to regional surface waters) and the absence of inorganic complexing agents. All media were made with 8 MΩ de-ionized water.

Medium (1)

$Ca(NO_3)_2$	1.2	10^{-3}M
$MgSO_4$	1.7	10^{-4}M
NaCl	3.4	10^{-4}M

pH 6.2

This medium was used previously (9, 11) for measurement of the mineral and organic pollutants toxicity on *Colpidium Campylum*.

(*) sufficient amount for.

The major drawbacks of this medium are the absence of buffering action and a pH which is too low for maintaining sufficiently stable complexes.

Medium (2)

This medium had the same composition as medium (1) but was buffered by 5. 10^{-4} M Borate-HCl buffer to reach pH 8.0.

Medium (3) [12]

$CaCl_2$	0.65	10^{-3} M
NaCl	2.5	10^{-4} M
$NaNO_3$	0.53	10^{-4} M
$MgSO_4$	3.07	10^{-4} M
Na_2SO_4	2.78	10^{-4} M
$NaHCO_3$	1.6	10^{-3} M

pH 8.15

This medium is more representative of the local surface water mineralization. It was previously used for toxicity studies (12) and allows the realization of long-term toxicity tests on Daphnia Magna (reproduction tests of 21 days).

Design of the Experiments

Factorial experiments were used for all these studies:

- Concentrations (C) of commercial and natural humic material (CH, NH) and cadmium used were increased in arithmetical progression.
- For calculation, reduced forms of (C) were used and calculated by the relation $c = \frac{C - \bar{C}}{\bar{C}}$, $\bar{C}$ being the mean of the arithmetical progression of concentration. This method gives orthogonal conditions for analysis and allows weighting of the coefficients obtained for each factor.
- The data were treated by a variance analysis; a mathematical model with a corrected specification (linear or quadratic equation) was calculated.
- The values of mean response surface and confidence intervals were calculated and the surface was represented graphically.

The design and statistical calculations were achieved with a special computer program which followed the methods of Snedecor and Cochran (13).

RESULTS

INFLUENCE OF THE MEDIUM ON TOXICITY OF CADMIUM - HUMIC MATERIAL (CH) SOLUTIONS

Influence of the Medium on Global Toxicity

The mean response surface obtained in the three media is illustrated in Fig. 1. The numerical values for C. Campylum growth calculated and the confidence intervals obtained by the equation are represented in Table 1. The whole data are, in the three media, the results of three experiments in which each combination of cadmium and humic material was tested in triplicate.

Table 2 gives regression equation coefficents for the relationship between cadmium and humic material their standard deviation and significance in t. test.

When different media are used, the same concentrations of cadmium and commercial humic material lead to various toxic effects.

In Table 1, one can note significant differences, even in the absence of humic material.

The examination of the regression coefficient of Table 2 shows that cadmium toxicity increases in the order: medium (1) < medium (3) < medium (2). A significant stimulation of growth by humic material is observed in the three media. There is a significant antagonism between cadmium and humic material in media (2) and (3). In medium (1), the toxic threshold of cadmium is scarcely reached at the highest tested concentration (60 $\mu g \cdot ^{-1}$); that is why no marked antagonism can be found.

Influence on Cadmium Complexation by Commercial Humic Material

Fig. 2 shows the free cadmium found in the three media in the presence of 7.5 $mg \cdot l^{-1}$ of CH in relation to total cadmium added initially.

It is obvious that in media (1) and (2), there is less free cadmium than in medium (3).

We have tabulated in Fig.3 the growth of Colpidium Campylum (in % of the control) as a function of the concentration of free cadmium in the medium. In medium (2), the presence of borate increases toxicity of cadmium for Colpidium Campylum at Cd concentrations above 26 $\mu g \cdot l^{-1}$. In medium (3), the toxic threshold is found to be between 40 and 60 μg^{-1}.

This preliminary study allowed the selection of the experimental conditions for further studies.

Medium (3) was selected because it had very similar properties to surface water and it gave good response surfaces data. The complexation of cadmium, which was lower in this medium than in medium (1) and (2) was probably more representative of environmental conditions.

COMPLEXATION AND TOXICITY OF CADMIUM IN RELATION TO THE NATURE OF HUMIC MATERIAL

Polarographic Studies

Figure 4 represents the level of free cadmium in various samples as a function of total cadmium added and concentration of humic material. One can note that the natural humic material has a higher complexing effect than did the commercial.

Ecotoxicity Studies

Table 3 gives the coefficients of surface response obtained in the presence of the two humic materials, CH and NH (data from two experiments in which each mixture was tested in triplicate).

The coefficients show that cadmium is apparently less toxic in the presence of NH than in presence of CH. A stimulating effect in the absence of cadmium more significant with CH than with NH can again be observed.

The growth of Colpidium Campylum related to free cadmium present in the medium (Fig.5) shows that the growth response of the ciliate is in direct relation with the concentration of free cadmium and not with the nature of humic material.

DISCUSSION

Influence of the Medium

The results of the present investigation show that both toxicity and complexation of cadmium are highly dependent on the compostiion of the medium. In medium 1, which is not buffered and has a low pH, cadmium is both more complexed and less toxic. The results are quite in agreement with previous measurements by Dive and Leclerc. if one takes into account the difference in experimental protocol (11).

During the work, we studied the complexing capacity of humic material in different conditions of water composition and not the direct complexation of cadmium by the medium components.

Many causes can explain the difference of complexation capacity of humic material between media 1 and 3. The ionic strengths are 4.6 mOsm in medium 1 and 6 mOsm in medium 3. The pH is very different, 6, 2 and 8, 15 respectively in medium 1 and 3. The ionic composition of media can influence the formation and stability of cadmium complexes, and the cadmium complexation kinetic, which was studied previously (unpublished results).

In the present experimental conditions, it is unlikely that precipitation of Cd can occur in presence of high molecular weight humic material because both cadmium and humic material concentrations are very low and the contact time and pH are not in the range which is optimal to induce precipitation.

The difference of growth observed as a function of the free cadmium concentration in media 1 and 2 emphasizes clearly the synergistic effect of borate on cadmium toxicity.

Influence of Humic Material

Humic materials are well known to have a complexing action on metal ions. But the ecotoxicological results of the complexation of heavy metals have not yet been studied extensively. The toxicity of cadmium in long-term exposure on Daphnia Magna decreases in the presence of humic materials (Erb et al., unpublished results).

Our results clearly show that Colpidium Campylum growth inhibition is directly related to the concentration of free cadmium in the medium.

Examination of equation coefficients obtained shows that the different effects observed between CH and NH are not present at the level of the interaction coefficients (Cd*CH or Cd*NH) which are very similar. On the contrary, the coefficients linked to cadmium (Cd and Cd*Cd) are strikingly lowered in the presence of natural humic material. These results imply that in the conditions of the experiment, the major part of cadmium fixed on humic material cannot be released into the medium. So the coefficient, linked to cadmium in the equation does not correspond to a lower toxicity of this metal, but to a smaller amount available in medium to exert a toxic effect on Ciliates.

Ecological Consequences

In natural waters, the concentration of humic material is highly dependent on the environmental situation (forest ...) and on the nature of aquatic systems (river, lake, or pond). Mean values usually found are about 10 $mg \cdot l^{-1}$. Even in polluted waters, cadmium rarely reaches very high concentrations. Anyway, it is possible that in local conditions (industrial wastes discharge) cadmium concentration can rise to the levels used in this study.

In these conditions, it appears that Ciliate Protozoa can be influenced both by cadmium contamination and by complexation phenomena which occurs in waters.

The above results clearly demonstrate that Colpidium Campylum can be considered as an advantageous model because it can detect the cadmium and complexation phenomena in a range of concentrations very close to natural conditions, whereas no significant effect is observed with the Daphnia test.

CONCLUSION

Toxicity tests using Colpidium Campylum with mixtures of cadmium and humic material are quite in agreement with polarographic measurements and show that Ciliate response is directly proportional to the concentration of free metal. In addition, the Protozoan test has emphasized the influence of the chemical composition of the test water on the toxicity of cadmium. The use of the Colpidium test associated with factorial experiments looks very promising for the study of ecotoxicological behaviour of metals in the environment. In natural conditions, Protozoan populations are probably affected by local concentrations of cadmium except that other metals are not present at significant levels. Indeed, the effect of metals associations on Ciliates is not yet known, except in some limited studies in the marine environment.

The complexation of cadmium by humic material can be significant not only in surface water aspects of pollution but also in the public health aspect of drinking water. During the purification process of surface water, the fraction of smallest molecules of humic material persists in water at the end of the treatment.

However, this fraction has the highest complexation capacity for metals.

So, in drinking water, cadmium micropollution can be carried by these low weight molecules and health consequences cannot yet, in any case, be predicted. Complementary studies will permit an appreciation of the hazard represented by water micropollution resulting from heavy metals.

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HUMIC MATTERS CONCENTRATION (mg.l^{-1})	MEDIUM	CADMIUM CONCENTRATION (µg.l^{-1})			
		0	20	40	60
0	1	100.2 ± 3.9	101.4 ± 2.9	98.6 ± 2.9	91.6 ± 3.9
	2	108.4 ± 7.8	106.7 ± 5.8	83.5 ± 5.8	38.8 ± 7.8
	3	100.2 ± 4.1	97.3 ± 3.1	85.4 ± 3.1	64.6 ± 4.1
2,5	1	105.3 ± 2.9	106.8 ± 2.3	104.1 ± 2.3	97.3 ± 2.9
	2	108.4 ± 5.8	109.4 ± 4.6	88.9 ± 4.6	46.9 ± 5.8
	3	100.3 ± 3.1	99.8 ± 2.4	90.2 ± 2.4	71.7 ± 3.1
5	1	109.1 ± 2.9	110.8 ± 2.3	108.3 ± 2.3	101.8 ± 2.88
	2	109.8 ± 5.8	113.4 ± 4.6	95.5 ± 4.6	56.2 ± 5.8
	3	100.2 ± 3.1	102. ± 2.4	94.8 ± 2.4	78.5 ± 3.1
7,5	1	111.6 ± 3.9	113.5 ± 2.88	111.3 ± 2.9	105. ± 3.9
	2	112.5 ± 7.8	118.7 ± 5.8	103.5 ± 5.8	66.8 ± 7.8
	3	99.8 ± 4.1	103.9 ± 3.1	99. ± 3.1	85.1 ± 4.1

- TABLE I -

Numerical values and confidence intervals of surface responses of Colpidium Campylum in presence of cadmium and humic matters in three different media.

MEDIUM	COEFFICIENTS OF SURFACE RESPONSE						
	Bo	Cd	CH	Cd^2	CH^2	Cd*CH	r^2
1	108.1	-3.8 ± 0.8 t = 4.64	+6.2 ± 0.8 t = 7.4	-4.6 ± 1.4 t = 3.2	-1.4 ± 1.4 t = 1.	+0.5 ± 1.1 t = 0.44	0.32
2	104.3	-28.8 ± 1.7 t = 17.1	+8.0 ± 1.7 t = 4.8	-24.2 ± 2.8 t = 8.6	+1.4 ± 2.8 t = 0.5	+5.9 ± 2.25 t = 2.6	0.81
3	97.9	-12.6 ± 0.9 t = 14.1	+5.0 ±0.9 t = 5.7	-10.1 ± 1.5 t = 6.8	-0.3 ± 1.5 t = 0.21	+5.2 ± 1.2 t = 4.44	0.68

- TABLE II -

Regression coefficients and standard deviations of surface responses of Colpidium Campylum growth in presence of cadmium and humic matters in three media (all data are in reduced expression of Cd and CH concentrations).

NATURE OF HUMIC MATTERS	COEFFICIENTS OF SURFACE RESPONSE						
	Bo	Cd	HM	Cd^2	HM^2	Cd*HM	r^2
CH	108.5	-10.2 ± 0.6 t = 15.9	+5.7 ± 0.64 t = 8.9	-12.5 ± 1.07 t = 11.6	-4.5 ± 1.1 t = 4.2	+2.3 ± 0.8 t = 2.7	0.92
NH	98.	-7.4 ± 0.39 t = 19	+1.0 ± 0.39 t = 2.6	-5.4 ± 0.65 t = 8.2	-1.7 ± 0.65 t = 2.6	+2.3 ± 0.5 t = 4.5	0.91

- TABLE III -

Coefficients and standard deviations of growth surface responses obtained with *Colpidium Campylum* in presence of cadmium and commercial (CH) or natural (NH) humic matters (HM).

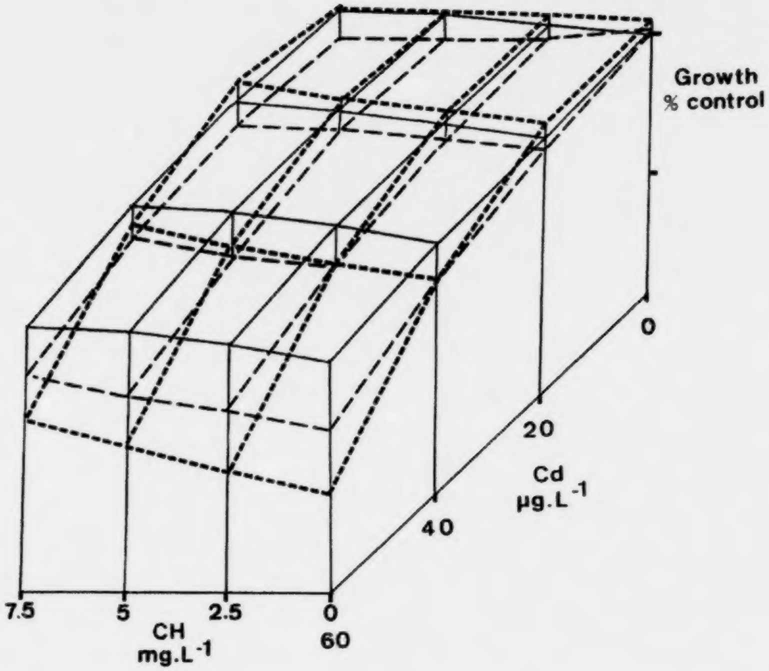

Fig. 1. Graphic representative of surface responses of Colpidium Campylum growth in presence of cadmium and humic matters in the three different media :

medium ① ——
medium ② -----
medium ③ — —

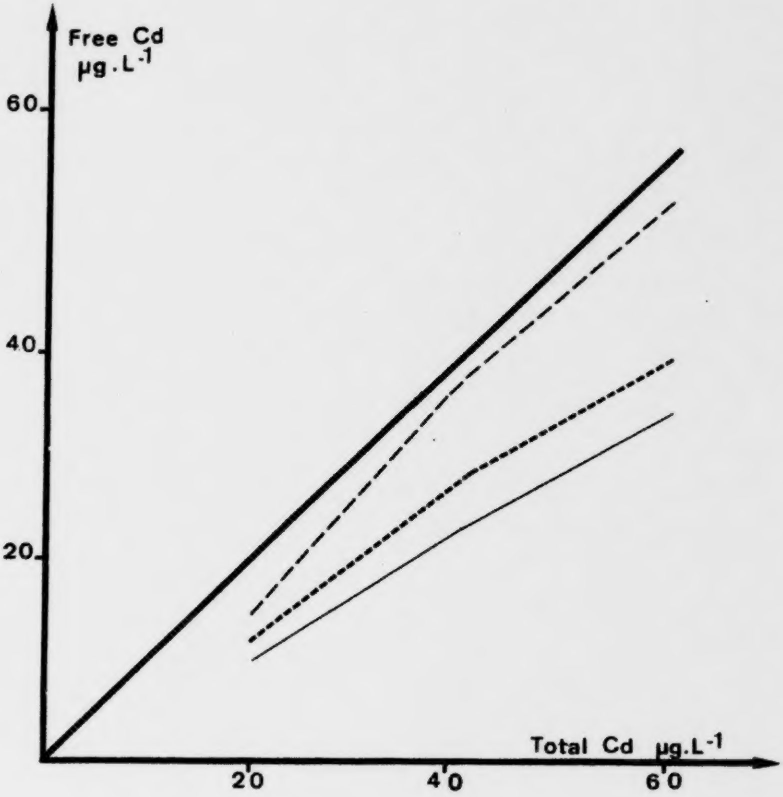

Fig. 2. Complexation of cadmium in presence of commercial humic matters (7.5 mg.l^{-1}) in different media :

medium ① ——
medium ② -----
medium ③ — —

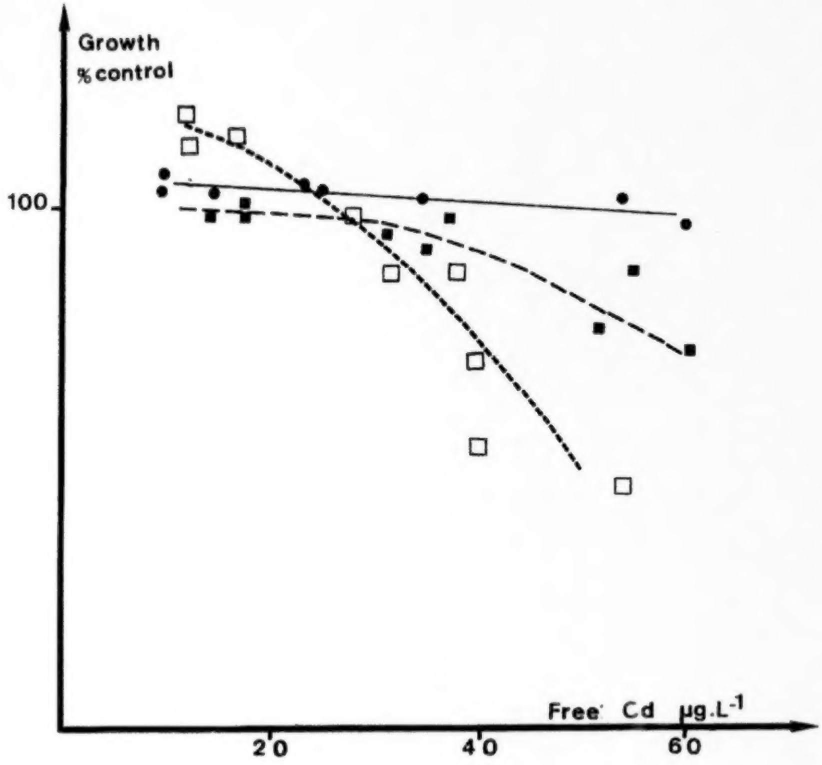

Fig. 3. Inhibition of Colpidium Campylum growth in function of free cadmium in three media :

medium ① —— ●
medium ② • ---- □
medium ③ — — ■

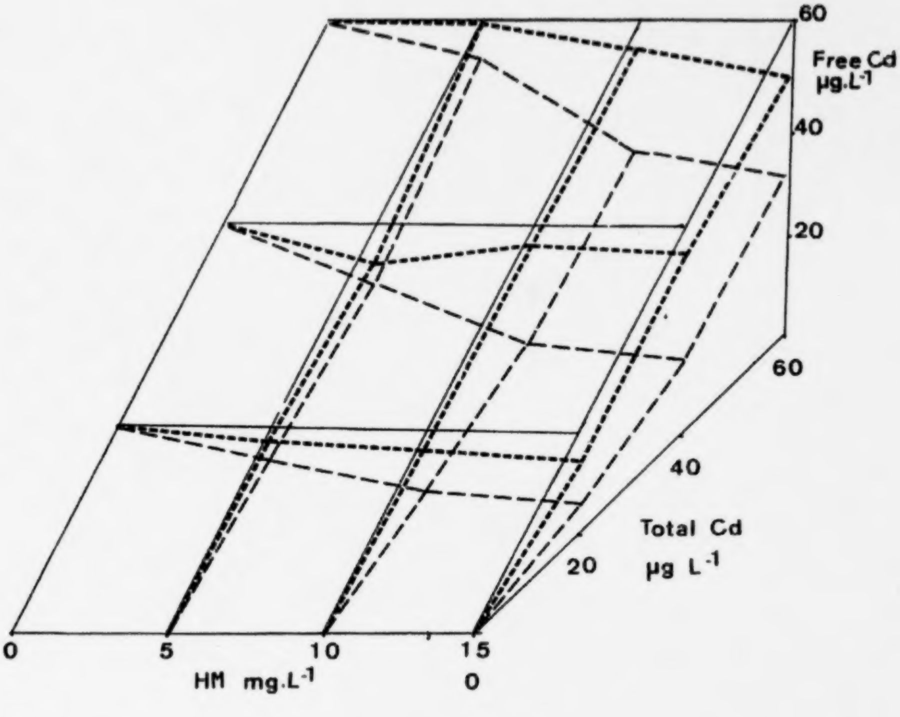

Fig. 4. Free cadmium present in samples in function of total cadmium added and concentration of commercial (----) and natural (— — —) humic matters.

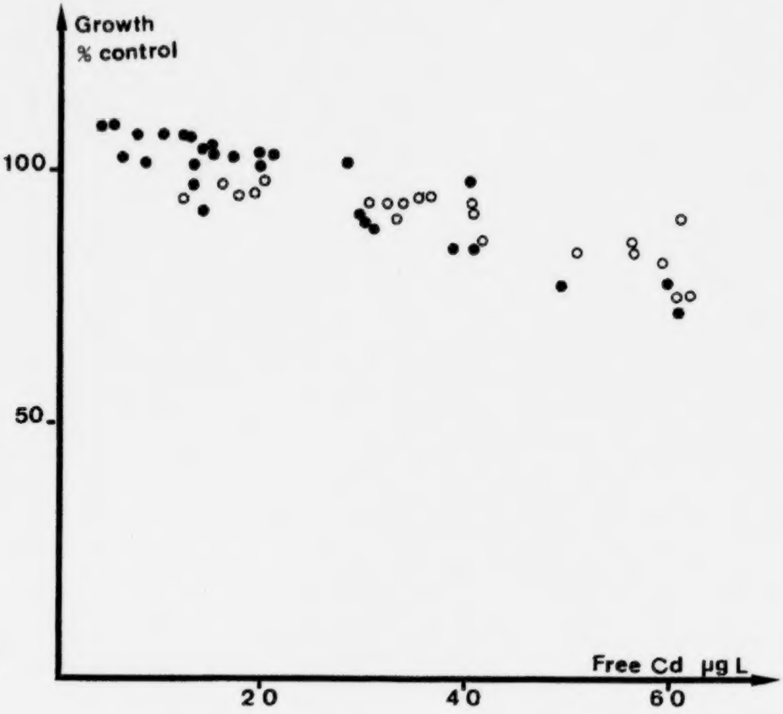

Fig. 5. Growth of Colpidium Campylum in function of free cadmium in presence of commercial (●) and natural (○) humic matters.

REGULATING TOXIC EFFLUENTS BY ELECTROPLATING INDUSTRIES IN ONTARIO: A CASE STUDY

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DWIVEDI, O.P. and T.C. McINTYRE. 1982. Regulating toxic effluents by electroplating industries in Ontario: A case study. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

This paper examines the process by which the Government of Ontario both regulates and monitors the production and use of effluents generated by electroplating industries in Ontario. The paper discusses: nature and scope of the problem including survey of the industry and effluent produced with its impact on the environment; management strategy for effluent control; effectiveness of the monitoring and surveillance system; and jurisdictional and international conflicts affecting the regulatory process. It concludes by raising the broader issue of accountable environmental management in Ontario.

Key Words: Electroplating, regulatory process, environmental management, industrial effluent.

DWIVEDI, O.P. and T.C. McINTYRE. 1982. La réglementation des effluents toxiques des industries de galvanisation en Ontario -- Une étude de cas. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Cette présentation étudie le mécanisme au moyen duquel le gouvernement de l'Ontario réglemente et contrôle la production et l'utilisation des effluents produits par les industries de galvanisation en Ontario. Y sont discutés: la nature et l'ampleur du problème, y compris un examen de l'industrie et des effluents et leur effet sur l'environnement; les méthodes de gestion employées pour contrôler la production d'effluents; l'efficacité du système de surveillance et de contrôle et les conflits juridictionnels à l'échelle nationale et internationale qui peuvent influer sur la réglementation. On invite, en terminant, à une gestion responsable de l'environnement en Ontario.

INTRODUCTION

Effluents resulting from the metal finishing activity of the electroplating industries have long been considered as one of the most serious water pollution problems. The problem is not so much of the volume of effluent discharged in the body of water (either directly or through the municipal sewage system), as it is the harmful impact of the heavy metals and other chemicals such as cyanide on water courses. The metal finishing process relates to the application of a mechanical, physical, chemical, or electrochemical operation to a metal surface to change its physical appearance, and to make it resistant to rust and corroding. It also applies to the application of a metallic coating to a non-metallic surface, such as plastic products. Included in the above process are such operations as cleaning, polishing, grinding, painting, phosphating, chromating, cyanide hardening, anodizing, electroplating, and metal finish hardening. These operations require the use of many chemicals, heavy metals, organic additives, solvents, oils and inert solids. Table 1 provides a classification of various types of these materials used in the metal finishing process is quite significant. But the most significant in terms of quantity and associated polluting effects are the toxic heavy metals, the toxic anions (such as cyanide) acids and alkalis, oils and oil emulsions, and biologically toxic or inhibitory complex-forming agents [1].

In a national survey done by Environment Canada in 1975, approximately 340 electroplating plants were identified [2]. Of these, about 65% were located in the Province of Ontario. But in terms of volume of production, over 80% of the industry was found to be in Ontario. The situation has not significantly changed since 1975 as evident from information one can obtain about the industry from business listing of industries by Dunn and Bradstreet Directory for the year 1981 [3]. (See Table 2) Within the Province of Ontario, nearly all industries are discharging effluents which end up in one of the two lakes - Ontario and Erie. Thus, it is important to concentrate on the study of toxic effluents by electroplating industries in the Province of Ontario rather than attempt a national study. Moreover, the largest section of the electroplating industry serves the needs of the automotive industry whose domestic sector is concentrated again in Ontario. The metal finishing operations are also required in power transmission, aircraft industries, appliance and furniture industries which are greatly concentrated in the golden horseshoe area of Ontario.

Table 3 illustrates the consumption pattern of chemicals by the industry in Canada as a whole (although this information pertains to the year 1977, the data are still relevant). Also illustrative, but not so up to date is the information given in Table 4 which indicates (as of 1977), the amount of metal and cyanide loads as effluents which could be expected from the electroplating industry in Canada. However, such a loading has been greatly reduced, especially in the Province of Ontario as cyanide treatment is required before discharge to sewage is made [4], and pH control as specified by municipal bylaws is supposed to be implemented. Such loadings give rise to serious environment problems throughout the Province of Ontario regarding their storage, handling, transporting, and eventual disposal (both through the municipal sewage system and through the dumping sites). These concerns have been examined below.

ENVIRONMENTAL IMPACT OF ELECTROPLATING INDUSTRIES IN ONTARIO

This section focusses on the treatment of wastewater and sludges resulting from the metal finishing industry, including the potential toxicological effects of several of the chemicals and heavy metals used in the finishing industry.

1. Wastewaters from metal finishing plants contain heavy metals, cyanides, and other inorganic substances. These constituents enter the effluent stream mainly through illegal dumps, spillages, leaks and rinse water, or from municipal sewage sludge applied incorrectly to land.
2. Dumps result from the disposal of spent solutions and sludges generated by the normal operations of a metal finishing plant. They may contain up to 200,000 mg/L of contaminants.
3. Dumping is usually an off-shift operation. Where generation is not practical, dumps can be segregated, treated or sent to a disposal facility.
4. Most of the chemical constituents, not biodegradable, are persistent in the environment, and tend to accumulate through the biological cycle.
5. Additional treatment methods have not yet been sufficiently developed to improve the quality of the effluent prior to discharge.
6. While centralized treatment still appears for disposal of spent baths and sludges, there has been significant development in this direction.
7. All but 9 plants of the industry discharge to municipal sewers of Ontario. The ineffectiveness of treatment as municipal plants requires many such discharges to be regarded as indirect discharges to water bodies.
8. Some new plants are still being installed in Canada with little or no consideration given to pollution control (not, however, in the Province of Ontario).
9. When discharge is made to municipal sewers, there is considerable variation in the quality of the eventual discharge to a water body for a number of reasons:

a) variation in removal of metals depending on the operating condition of sewage treatment plants and the particular metal concerned.
b) possibility of by-passing sewage treatment during heavy rainfall.

Sludge containing inert heavy metals as well as other by-products not dispersed into wastewater also accounts for a fairly large percentage of liquid industrial effluent generated in Ontario as well as generated disposal problems [5].

Potential Environmental Impact and Deleterious Nature of Wastes Produced by Metal Finishing Industry

Several of the compounds used in the electroplating industry pose difficult problems in disposal. Many are classified as toxic wastes - "discarded material or combinations of which cannot safely and adequately be handled by existing waste management facilities because they pose a substantial present or potential hazard to human health, or other living organisms or the physical environment because of their chemical, biological, or physical properties." [6] These wastes can be categorized under the following headings (each requiring special handling, storage, and disposal methods): ignitable, corrosive, reactive, toxic, infectious, bioaccumulative, mutagenic, carcinogenic, and teratogenic.

As a large percentage of metal finishing facilities are discharging to the watershed of the Ontario and Erie lakes, our analysis will shift towards implications of this waste on the Great Lakes ecosystem. Several difficulties prevent the detection of impact on human health from these sources of waste in the Great Lakes. Most notable of these are problems associated with epidemeological studies, data collection, interactions of heavy metals, and multiple toxicity [7].

The impact of hazardous substances discharged into the Great Lakes depends not only on their toxicity but also the ways in which they move through the lakes. Transport and fate pertains to how chemicals will move through the lakes, how they will degrade, where they will reside for short and long periods, and how they will be removed from the system. Various chemicals exhibit different transport and fate characteristics as a result of their individual physical-chemical properties. These characteristics affect the surveillance approach needed to monitor the chemicals; the loading that results in various concentrations in water, sediment and biota; the control programs that are required; and the cost benefit ratios that can be expected from control efforts [8]. There are several obstacles to an adequate assessment of the relative importance of the various pathways for these hazardous substances entering into the Great Lakes. Which of the many thousands of chemicals produced along with these wastes in the basin should be considered? Which source emissions are most significant? Which should be given priority status? Are there adequate concentration data for the various services of hazardous waste in the basin upon which an assessment can be made? It is noted by the I.J.C. that estimates of hazardous substances loadings using general or national data bases and extrapolating to the Great Lakes basin are at best only first approximations. They can indicate the need and direction for specific monitoring to confirm loadings to provide a basis for regulatory or control programs [9]. Compounding the problems of assessing the loading of the Great Lake basin by wastewater and effluent, are those difficulties in determining the quantity and source of atmospheric emissions from the metal finisning industry.

Toxicity to Humans

The potential for exposure to a number of compounds used in the metal finishing process alone, but as well in their treatment and disposal. The manner in which these compounds are disposed of often determines the source as well as potential for deleterious effects to humans - whether it be from drinking water, contaminated fish, food from land treated with sludge, or the atmosphere. It is well documented that some of the industrial pollutants from the finishing industry are the major causes of irreversible damage to the biosphere. A recent issue of Cancer Research has emphasized this in a report of the International Agency for Research in Cancer (IARC) [10]. This report indicates that 18 or 54 chemicals used in industrial processes are human carcinogens. Included in this list are nickel, chromium, zinc, cadmium, and their compounds - all commonly utilized in metal finishing processes [11].

Chromium and various chromates have been known to cause chromosomal aberrations including sister chromatid exchange and unscheduled DNA synthesis in cultured mammalian cells, mutants to microbial systems, and cancer to man and animal [12]. Nickel dusts have been known to cause lung cancer in refinery workers, and nickel and chromium compounds have been implicated as contributing agents in the induction of cancer. Epidemiological studies have demonstrated increased incidences of cancer in the nasal cavity, lung, and possibly larynx in nickel workers [13]. Studies have suggested that occupational exposure to cadmium in some form increases the risk of prostate cancer in humans. Of critical importance is the fact that most of the chemical constituents are not biodegradable, are persistent in the environment, and tend to accumulate through the biological cycle. This has implications not only for man but for all components of the aquatic as well as the terrestrial environment. The foregoing discussion indicates the need for careful consideration in the usage, treatment and disposal of metal finishing by-products into the environment.

MANAGEMENT STRATEGY FOR EFFLUENT CONTROL

This section deals with some aspects of effluent control strategy and guidelines which have been formulated by the federal, provincial, and municipal authorities. Also included in the discussion is a brief review of technology development to control water pollution, and the impact of requiring abatement control on the finances of metal industries.

The Federal Government Response

Federal effluent regulations and guidelines for the industrial sector are based on the policy that national effluent requirements represent a minimum acceptable baseline level of effluent quality development on the basis of best practicable technology. All the guidelines and regulations under the Fisheries Act are developed by task forces consisting of representatives from Federal and Provincial governments and the industries concerned.

The Fisheries Act (Section 33 (2)) prohibits anyone from permitting the deposit of a deleterious substance in any place such that the deleterious substance may enter any water frequented by fish. Under the Canada-Ontario accord, Ontario has agreed to establish and enforce requirements at least as stringent as the agreed Federal baseline requirements and to conduct surveillance of effluents. The Federal effluent requirements will be applied at new and expanded production facilities and will be applied as a minimum as rapidly as possible in all other cases. The Federal Government has established effluent requirements for a number of industrial categories [4].

A government/industry task force was established in 1976 to study environmental aspects of the industry and propose appropriate control measures. The task force agreed that a combination of regulations/guidelines could be the best management strategy to control effluent of toxic wastes arising out of the electroplating process. A national control requirement system was developed which included the following items:

1. "Explicity prohibit dumpting activities.
2. Encourage good housekeeping practices to contain leaks, spillage, and accidental losses.
3. Limit the concentration of certain control parameters in the final effluent.
4. Provide adequate reporting procedures, to ensure that the requirements are met." [15].

Subsequently, national guidelines were formulated under the title, Metal Finishing Liquid Effluent Guidelines, and were published in the Canada Gazette on 5 November 1977 [16]. The guidelines suggested effluent should meet the following four criteria:

1. Effluent: A composite sample should meet have a pH between 6.0 and 9.5 and other substances should be limited as specified below:

Substance	Maximum Total Concentration in mg/L
Total Suspended Matter	30.0
Cadmium	1.5
Chromium (total)	1.0
Copper	1.0
Lead	1.5
Zinc	2.0
Nickel	2.0
Cyanide (oxidizable)	0.1
Cyanide (total)	3.0

2. Concentrated residues containing emulsion cleaners and chlorinated hydrocarbons, and effluent treatment sludges should not be deposited with the effluent.
3. Other concentrated spent processing solutions and residues should be treated to meet the effluent objectives or should be stored and disposed of in a manner which the Minister may consider acceptable.

In addition to these guidelines, a code of good housekeeping practices (EPSI-WP-77-5) was formulated. Together, these two apply as national baseline standards.

Provincial Guidelines

The Ontario provincial guidelines are laid down in the Objectives for the Control of Industrial Waste Discharges in Ontario. They are concerned with protection of receiving waters, desirable effluent discharges, suspended solids, toxic substances, methods of disposal, and contaminants in drinking water - to name a few [17]. In regard to heavy metals, the effluent concentration of component heavy metals shall not exceed the following limit as specified below:

Substance	Maximum Total Concentration in mg/L
Cadmium	0.001
Chromium	1.0
Copper	1.0
Lead	1.0
Mercury	0.001
Nickel	1.0
Tin	1.0
Zinc	1.0

Compared to the suggested national guidelines, the Province of Ontario has more stringent requirements of toxic effluent control. These guidelines, with the exception of about 9 plants, are then enforced by the municipalities under their specific bylaws. In the case of those plants which release their effluents directly to streams and are beyond the jurisdiction of municipal authorities, the Ministry of Environment is responsible for monitoring, surveillance, and enforcement.

Municipal Guidelines

In a survey conducted by Buffa and Coulter (1976), it was estimated that in the Province of Ontario, only 70% of the metal finishing plants discharge to municipal sewers. For other provinces, the percentage reported was [18]:

Atlantic Provinces	75%
Quebec	60%
Prairie Provinces	45%
British Columbia	50%

However, since that survey was done, the situation in Ontario has greatly improved. As of mid-1982, about 96% of electroplating industries discharge to municipal sanitary sewers [19]. (The remaining nine industries do discharge to water courses, but under constant surveillance of the OME.) And as sewage treatment facilities have been constructed and are in operation for all municipal areas which border the watershed area of the lower Great Lakes, and as the Great Lakes Water Quality Agreement specifically requires such sewage treatment facilities, and as about 96% of metal finishing industries are located within such watershed area, direct discharge to water courses is not evident. Very stringent controls, through municipal bylaws as required by MOE, exist. Therefore, while the possibility of indiscriminate discharge of untreated metal finishing waste may exist in other provincial jurisdictions, in Ontario the problem seems to be, by and large, under control. What happens sometimes is that if an industry tries to discharge a partially treated waste into the municipal sewer, a warning is given, and then a threat to close down the industry. As a matter of fact, several electroplating industries have gone out of business in the period of 1978-1981 partially because of slump in auto industry and partially due to stringent water and air pollution controls.

With regard to municipal sewer bylaws it can be said that these have been designed mostly to reach a required level of treatment rather than to protect aquatic plants and fish.

Historically, sewer bylaws have been designed to protect sewers and treatment plants, and not to protect fish in waters receiving municipal effluent [20]. The effectiveness of municipal treatment plants in the removal of metal finishing wastes is contingent upon a number of factors: characteristics of effluents, interactions with municipal sewage, and the types of sewage disposal plants in existence. Generally, it can be said that a sewage treatment plant has a limited ability to treat the wastes found in metal finishing plant effluents - its effectiveness may be high for the organic materials and much lower for metals dissolved in the effluent. Any analysis of effectiveness of any sewage treatment plant in removal of metal finishing wastes should be based on an analysis of the wastes and on unique technological capabilities of the sewage treatment plant.

It should be noted that while individual municipalities of Ontario have adhered to the suggested provincial guidelines for effluent control, there may very well be a variation in the enforcement technique. For example, if a new electroplating industry is being established in a municipal industrial area, chances are, in some areas, that the industry may know about effluent control guidelines after the plant has become operative. Obviously, there is a need not only to remove any variance in municipal bylaws but also to standardize the enforcement policies of municipal water pollution control programs. Consideration in policy direction should be aimed towards the reduction in several components of effluents of the metal finishing industry in addition to upgrading the effectiveness of several treatment plants throughout the province. In a survey conducted in 1978, it was found that, in a sample of 57 municipalities in Ontario, the electroplating related metals received about 50% removal efficiency. Although major improvements have since been made, it still gives us an uncomfortable feeling about the efficiency of municipal plants in Ontario in toto, and gives cause for serious concern as well as calling for a complete evaluation of existing treatment facilities. Arising out of this discussion is the great need for the upgrading of existing municipal treatment plants in addition to restricting the amounts of effluents which can be released by the metal finishing industry into the environment. The first step in the right direction are the new limits recommended by a joint committee of the Ministry of the Environment and Ontario Municipal Engineers Association for the City of Toronto. The new bylaw 2520 calls for concentration limits for heavy metals and other substances as well as regulating the discharge of wastewater. It is considered to be the most up-to-date and most stringent of all industrial waste control bylaws throughout the province. This is indeed appropriate as the City of Toronto and the greater Metro-Toronto area are responsible for over 30% of all metal finishing business (volume) in Canada. The new standards for discharge into both sanitary sewers as well as storm sewers can be seen in Tables 5 and 6. It is interesting to note here the potential for a confrontation between the metal finishing industry in Toronto and the Metropolitan Toronto Works Department, particularly when one takes into account the volume of waste generated in the area by this industry. However, much will depend upon the enforcement of this bylaw. In summary, concern over the aspect of "equity" has been expressed by the metal finishing industry in Ontario. This "equity" consideration relates to the different degrees of severity in enforcement of bylaws exercised by a number of municipalities throughout the province. Apparently, the desire to either attract or discourage the establishment of new metal finishing plants is reflected in the stringency of the enforcement of bylaws [21].

The above review of federal, provincial, and municipal effluent control guidelines illustrates the need to attack the problem from two directions. While stringent standards must be applied, at the same time the industry must be encouraged to consider the loss of chemicals and metals to sewers. Industries would be more co-operative if they are shown that in these days of hard economic times such a loss can be prevented; and by applying "Best Practicable Technology", the industry can still meet municipal requirements while keeping itself out of financial trouble.

Technology Development to Control Toxicity from Electroplating Process

The International Joint Commission, in its 1982 report, observed that monitoring and research functions are essential components of "maintaining and restoring the physical, chemical, and biological integrity of the Great Lakes Basin Ecosystem...Research should be intensified to determine the pathways, fate, and effects of toxic substances aimed at the protection of human health, fishery resources and wildlife of the Great Lakes Basin Ecosystem."[22] The IJC, in the earlier report (1980) suggested that in order to prevent pollution from the metal finishing industry, control alternatives should be considered which can be grouped into five major options: wise use, reuse, bans, treatment technology, and use of assimilative capacity [23]. Associated with each one of these alternatives is a corresponding cost. Historically, the wastewater costs were relatively modest and limited to: (1) cost of water itself; (2) cost of chemicals lost to wastewater; (3) occasionally, the cost of destroying or removing the more spectacular toxics such as cyanide and hexavalent chromium; and (4) where so situated, the cost of municipal sewer service [24]. Wastewater costs now include: (1) generally increased cost for the water itself; (2) the cost of process chemicals lost to wastewater; (3) the substantial investment cost for wastewater control facilities; (4) the operating expense for wastewater control - chemicals, labour, power, and maintenance; (5) the cost of skilled, technical supervision; and (6) where so situated, the increased charges for discharge of treated wastewater into a municipal sewer [25]. Equally as important as the treatment of this wastewater is the maintenance and prevention of accidental spills of chemicals to the effluent stream. To compound this situation, most of these accidental dumps occur offshift when there is a minimum of control in most installations in the handling of their disposal when the treatment system is not operating. Whether deliberate or accidental, the potential for detrimental damage to the environment of these discharges is quite high. In a report by Buffa and Coulter (1976), it was found that concentrations in these dumps and spillages may vary from 200,000 or more mg/L for chromic acid to 500 to 1000 mg/L for metal in nitric and sulphuric acid solutions [26]. In addition to the possibility of environmental degradation, the obvious cost factors in chemical losses to the metal finishing firms need to be considered. Regarding accidental chemicals lost, perhaps the industry was not as concerned about it in the past; however, during the late seventies, individual metal finishing companies began "to view the loss of chemicals and metals to sewers as an economic disbenefit and have acted accordingly." [27] The rising costs of chemicals and production have come as a boon in disguise for the industry since it is now economically beneficial to reduce chemical loss to sewers to a minimum.

Success in the areas of research and development to recover chemicals and metals resulting from finishing processes is contingent upon maintenance of a cost low enough to make the process economically viable, as well as the availability of a market for the materials recovered. Some inroads, as reported in 1975, which were considered good in the recovery of chemicals and metals are the following: cyanide destruction, electrolytic destruction, carbon absorption and catalytic oxidation, chromium reduction and removal, and precipitation of metals as hydroxides [28]. Rarely are any of these or the aforementioned methods of recovery found alone but in combinations of two or more systems. The determination of the system used is dependent upon the various cost factors of labour, materials, space, plus the skill and knowledge of available personnel. Of the above methods, metal precipitation cannot now be considered a panacea mainly because of increasing costs of chemicals and waste sludge disposal.

With respect to the control of sludges, these are generated in four ways in the metal finishing industry: filter cleanouts, bath purification, burnishing and barrel discharges, cleaning solutions, and metallic hydroxides from chemical waste treatment processes [29]. One of the problems resulting from earlier mentioned treatment of metal finishing wastes is the formation of relatively large quantities of residual sludge. The sludge presents difficulties in disposal, has limited value because of the relatively low concentrations of useful materials it contains, and by the processes which cause its formation, constitutes an expense [30]. Most of Ontario's liquid industrial wastes are currently treated and/or disposed of by one or more of the following methods [31]

(a) controlled discharge to municipal sewage systems; (b) co-disposal with solid wastes in some 20 to 25 landfills; (c) product recovery with incineration of the residues; (d) export to facilities in United States (est. 500,000 gals/month); (e) incineration of non-halogented organics at the Tricil Ltd. plant near Corunna; (f) some chemical fixation in Hamilton, Ontario, by Frontenac Chemicals Ltd.; and (g) some suspected illegal dumping (quantity believed to be limited). Metal finishing wastes alone accounted for approximately 3 million gallons of industrial waste generated in the Province of Ontario in 1979.

Other developments in the field of research and technology for pollution prevention and control have been detailed by the Environment Canada in its 1982 report [32]. A Wastewater Technology Centre is being operated by the Environmental Protection Service of Environment Canada in Burlington, Ontario. Some specific companies have been helped by EPS. Through a funding mechanism in conducting research, some of these projects are briefly described below [33].

1. The PARR System: It makes extensive use of automated reciprocating flow in exchange units to recover chromic acid, nickel sulphate and copper sulphate from process rinses. Evaporators are used either to concentrate the product or to create space in the process tanks to add the recovered product. At the same time, the purified water is returned to the rinse tanks for reuse. A small conventional waste treatment system consisting of chrome reduction, neutralization, precipitation clarification, and a filter press is employed for treatment of residual wastewater from the recovery units.
2. HSA Metal Recovery System: The HSA reactor works on the principle of electro-chemical recovery of metals on high surface area (HSA) modules of carbon-fibres set in a patented reactor design. It has been found that the process has the capability of recovering cadmium from the plating bath as well as destroying cyanide. The process was tested by the staff of the Wastewater Technology Centre, Burlington, under both simulated and actual operating conditions in an industry located in Burlington.
3. Chrome - Napper System: In chrome plating operations, up to 90% of the chrome added to the plating baths may be lost to plant drains. Therefore, the wastewater requires treatment before discharge to city sewers. But through the use of a new membrane technology called the "chrome-Napper" system, chromium is recovered and can be reused, and there is virtual elimination of chrome-bearing discharges to sewers. The technology developed by the Innova Corporation, Clearwater, Florida, consists of an ion-transfer module which permits the ionic transfer of chromates and sulphates through a polyester-based membrane to an anode compartment where the chemicals concentrate prior to return to the plating bath. The membrane is enclosed in a stainless steel mesh which acts as the cathode while inside membrane is a platinum-plated titanium anode. A DC current across the cell electrodes encourages the passage of ions through the membrane. The equipment is being installed in Canada in a metal finishing industry specializing in nickel-chrome plating.
4. SO$_2$/Air Oxidation Process: INCO Research has patented process for the destruction of cyanide contained in wastewaters. The process involves the oxidation of cyanide with sulphur dioxide and air in the presence of a copper catalyst and seems to destroy all cyanide forms including iron cyanide complexes. The destruction of metal cyanide complexes results in the formation of metal hydroxides from which precipitate are removed.

In addition to these research and technological developments, efforts are being made, in co-operation with the industry, to control leaching of toxic chemicals through wastes. Research efforts by the EPS Wastewater Technology Centre are commendable.

Present Waste Management Practices

Presently, most industrial wastes are removed from metal finishing facilities by independent truckers. However, this is expected to change due to increases in haulage costs. The bulk of Ontario industrial wastes are generated in the following areas:

a) Windsor, Sarnia, London, and surrounding areas;
b) Kitchener-Guelph-Cambridge triangle; and
c) the Golden Horseshoe from Oshawa to Niagara Falls

The major concerns with the present practice of waste disposal encompass these parameters (34):

i) Most of the province's industrial wastes are co-disposed in a number of sanitary landfills posing a threat to local ground water regimes.
ii) 10% of the province's waste is now hauled across the border to Ohio, Michigan and New York for treatment or disposal. There is no guarantee that these borders will remain open for any indefinite period.
iii) There are no "high temperature" incinerators in Canada for destroying a variety of these wastes.
iv) For a variety of valid reasons, the private sector has not provided the necessary range of facilities to properly manage all wastes geneated in the province.

Apart from new technology required to store, treat or destroy the wastes generated from the metal finishing industry in Ontario, the following facilities are felt to be urgently required to ensure proper management of vuture wastes produced from this source [35]: (i) a secure landfill site for disposal of compounds plus residues from pollution abatement facilities (particularly sludge and wastes that have been chemically fixed); (ii) a physical chemical treatment complex where wastes can be neutralized and/or destroyed; (iii) a high temperature incinerator with appropriate emission control devices; (iv) collection/storage depots for Northern and Eastern Ontario.

A management strategy devised by the Ontario government to both monitor and control existing waste management disposal practices was announced in October 1975. The program is to ensure that the methods and facilities used in transportation, treatment and disposal of liquid industrial wastes provide no threat to human health or the environment. However, in addition to the above, what is needed now are "legislative patterns designed to encourage this positive approach to pollution control, instead of present ones, which are in the main merely restrictive in nature and always potentially productive of schemes between the regulatory authorities and industry." [36]

Financial Impact of Pollution Abatement of Metal Finishing Industry in Ontario

No discussion of assessment of pollution control for the metal finishing industries would be complete without a note on the financial implications it may incur. In response to the proposed Federal Guidelines for the metal finishing industry, representatives of the industry expressed the following concerns [37]:

a) Government assistance should be available to help finance pollution control equipment at less than commercial rates.
b) Controls must be applied equally throughout the industry. The necessity for control was recognized but its lack of uniform application in the past has caused hardship to some companies.
c) New jobbing plants in the medium sized category or smaller, would be discouraged by the capital costs of waste treatment indicated.
d) Alternatives to metal finishing, such as powder plastics, or stainless steel, would not seriously affect their ability to increase prices to cover the cost of waste treatment.
e) There has been one instance in the Toronto area where the cost for waste treatment was given as the reason for closing out an operation. (Similar instances have occurred in other areas of Ontario in 1980 and 1981).

A number of factors were taken into account to assess the possibility of plant closures due to an economic inability of some plants to meet stringent pollution control guidelines, and also due to hard economic times.

It is certain that without government help, the metal finishing industry cannot hope to reach the level of federal and provincial guidelines; otherwise, considering the highly competitive nature of the industry and resulting increases in production costs (due to installation of new and costly pollution control equipment, which in turn will increase prices) some plants may be closed and the business could shift to outside of Ontario or Canada.

CONCLUDING OBSERVATIONS

Attempts have been made in this paper to present a brief overview of the metal finishing industry in the Province of Ontario. Divergency in locations and size of plant operations, diverse production processes, and the system surveillance and enforcement of guidelines all contribute to a number of problems facing this industry today, particularly in terms of environmental protection. Policy directed towards control and regulation of effluent from this industry must be both "a rudder and a sail" in terms of the existing metal finishing industry. There is an emergent need for all parties concerned to help in developing and maintaining a long-term perspective on the environmental quality, and the preservation and protection of the environment of Ontario. Toward this end, it will be desirable to encourage a non-adversarial attitude between those who regulate and enforce measures and those who manage the industry. At the same time, federal-provincial co-operation, and co-ordination in standards-formulation and in the development of technology for waste water and sludge control is extremely important. As noted by the IJC, (although remarks were directed to the two federal governments of Canada and USA), overall management of toxic substances control programs is not properly facilitated.
"... There has been insufficient co-ordination of activities within programs. This fragmentation has resulted in duplicate activities in some cases, incomplete program coverage in others, and a limited management capacity to effectively address emerging complex problems." [38]

Some recommendations are offered as a part of our concluding remarks:

1. While the Ontario Ministry of Environment and the municipalities have stepped up their degree of enforcement (of control-requirements), variations do exist. It is necessary to have uniformity in regulatory activity. Toward this end, the MOE should consider monitoring and spot-checking options.

2. Increased emphasis should be placed on the disposal of toxic wastes, including the surveillance of transportation, dumping, and removal of such wastes. Disposal should include transportation of the waste,

and should cover the movement inside Ontario, as well as the cartage to some designated dump-sites in the USA.

3. Control requirements should be based or expressed as objectives for a limited amount of time to give the parties involved sufficient lead time to organize and meet these standards.
4. Research and development efforts must be coordinated between the federal and provincial governments, and industry should be encouraged to invest in such efforts.
5. There is a need for centralized waste facilities to handle the sludge and spent chemicals from the industry although every effort should be made to solve the problems of treatment and disposal at the source.
6. A task force composed of Federal/Provincial/Municipal environmental representatives and metal finishing industry representatives should be established to study and report on the issue.
7. There is a need to have a national depository of information consisting of:

   a) size, volume, and location of this industry throughout the province to facilitate legislative and maintenance processes; and
   b) handling, storage, treatment, and removal of hazardous and toxic substances which result from these processes.

8. Companies should be required to supply evidence of treatment either within the existing facilities or outside the facilities, and every effort should be made to record and monitor the movement of effluent from this industry to its final disposal site, along every step of handling and transportation processes.
9. The metal finishing industry in Canada needs a voice to act as a responsible corporate body. At present, there is no such organization of the industry, although the Automotive Parts Manufacturer's Association does try to represent a specific segment of the industry. Such an association of the metal finishing industries in Canada should, then, establish an advisory board composed of industry managers and outside independent researchers to advise the industry on the question of appropriate technology, a code of conduct toward reducing to the maximum extent practicable the discharge of toxic substances, and ways and means to interact effectively with the federal, provincial, and municipal authorities in Canada.

These recommendations (not exhaustive at all) are both initiative and responsible to ameliorate some of the problems facing the metal finishing industry in Ontario. By adhering to them, it is felt that the industry can remain a viable one and continue to operate with a minimum amount of economic and environmental disruption.

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TABLE 1 CLASSIFICATION OF TYPES OF DISCHARGE FROM SURFACE TREATMENT PROCESSES

TYPE	EXAMPLES
Acids	HCl, H_2SO_4, H_3PO_4 (and acid phosphates), HF, H_3BO_3, (often discharged with dissolved heavy metals present)
Alkalis	NaOH, Na_2CO_3 (frequently with phosphates, silicates and detergents, and often containing oils and oil emulsions)
Heavy metals in solution	Cd, Co, Cr, Cu, Fe, Mo, Mn, Ni, Pb, Sb, Sn, Zn.
Complex-forming agents	CN^-, amines, NH_3, EDTA, NTA, citrate, tartrate, oxalate, gluconate.
Organic additives	Aldehydes, ketones, alcohols, fatty and aromatic carboxylic acids, carbohydrates, sulphonic acids, dyes, phenols.
Solvents	Trichlorethylene, toluene, xylene, alcohols.
Oils, waxes, and greases	Sometimes discharged with detergents in emulsified form.
Inert solids	Grinding materials, (oxides, carborundum, etc.)

Source: Fisheries and Environment Canada (1977) "Recovery and Re-use of Metals and Chemicals From Metal Finishing Wastes" in Waste Handling, Disposal and Recovery in the Metal Finishing Industry, EPS-3-WP-77-3, p. 47.

TABLE 2 METAL FINISHING INDUSTRY SIZE AND GEOGRAPHICAL DISTRIBUTION - 1981

	No. of Companies	No. of Employees No.	%	Sales Amount	%
Atlantic Provinces	2	21	0.3	$ 755,000	0.2
Quebec	64	1723	23.9	62,068,000	14.0
Ontario	169	4080	56.5	327,200,700	74.0
Manitoba, Saskatchewan and Alberta	39	682	9.4	24,498,000	5.5
British Columbia	16	715	9.9	27,748,000	6.3
Total Canada	290	7221	100.0	$442,269,000	100.00

Source: computed from Gore and Storrie Limited, Canadian National Inventory of Hazardous Wastes, (a report prepared for the Environmental Protection Service, Environment Canada), January 1982, Volume I, SIC Group 3471. Note that not all metal finishing industries were included in the data collected by Dunnand Bradstreet, Directory of business listing which forms the basis of above report.

TABLE 3 CONSUMPTION OF CHEMICALS BY THE METAL FINISHING INDUSTRY (kg/yr)

Cadmium Compounds (Including Cyanides)	11,612
Copper Compounds (Including Cyanides)	201,852
HCl, H_2SO_4 and Other Acids	10,055,858
Chromic Acid and Miscellaneous Chromates	779,738
Phosphoric Acid and Miscellaneous Phosphates	2,133,281
Nickel Compounds	568,361
Caustic Soda	1,148,969
Other Cyanides	526,630
Detergents	981,590
Chlorinated Hydrocarbons	2,540,160
Alkali Cleaners	276,696
TOTAL	19,224,347

Source: Coulter, K. R., (1977) "Geographical Distribution of Metal Finishing Plants in Canada" in, Waste Handling, Disposal and Recovery in the Metal Finishing Industry, Fisheries and Environment Canada, EPS-3-WP-77-3. p. 114 (Quantities have been converted into SI units, and as such are not exact to original weight).

TABLE 4 METAL AND CYANIDE IONS IN THE WASTE LOAD OF ELECTROPLATING OPERATIONS IN CANADA

The chart below shows the total metal and cyanide ions loads that can be expected in the effluents from the electroplating industry, based on the consumption of the salts as reported in the survey of the industry.

Metal Ion	Rinses (kg)	Accidents Etc. (kg)	Total (kg)
Copper	21,773	66,226	87,999
Nickel	35,381	81,648	117,029
Chromium	142,430	126,101	268,531
Zinc	36,968	46,267	83,235
Cadmium	3,175	4,082	7,257
Cyanide Ion	42,638	99,792	142,430
		TOTAL	706,481

Source: Coulter, K. R., (1977) "Geographical Distribution of Metal Finishing Plants in Canada", in Waste Handling, Disposal and Recovery in the Metal Finishing Industry, Fisheries and Environment Canada, EPS-3-WP-77-3, p. 117. (Quantities have been converted into SI units, and as such are not exact to original weight).

TABLE 5

CITY OF TORONTO-PROPOSED NEW LIMITS FOR DISCHARGE INTO STORM SEWERS

MATTER	EXPRESSED AS	CONCENTRATION IN MILLIGRAMS PER LITRE
Aluminum	Al	1.0
Arsenic	As	1.0
Barium	Ba	1.0
Cadmium	Cd	0.1
Chlorine	Cl_2	1.0
Chromium	Cr	1.0
Copper	Cu	1.0
Cyanide	HCN	0.1
Flouride	F	2.0
Iron	Fe	1.0
Lead	Pb	1.0
Manganese	Mn	1.0
Mercury	Hg	0.001
Nickel	Ni	1.0
Phenolic compounds		0.02
Phosphorus	P	1.0
Suspended solids		15
Tin	Sn	1.0
Zinc	Zn	1.0

Source: City of Toronto By-law 2520, Engineering Department, Metropolitan Toronto, March 4, 1981.

TABLE 6

CITY OF TORONTO-PROPOSED NEW LIMITS FOR DISCHARGE INTO SANITARY SEWERS

MATTER	EXPRESSED AS	CONCENTRATION IN MILLIGRAMS PER LITRE
Aluminum	Al	50.
Arsenic	As	1.0
Barium	Ba	5.0
Cadmium	Cd	2.0
Chloride	Cl	1500.
Chromium	Cr	5.0
Copper	Cu	5.0
Cyanide	HCN	2.0
Fluoride	F	10.
Iron	Fe	50.
Lead	Pb	5.0
Mercury	Hg	0.1
Nickel	Ni	5.0
Phenolic compounds		1.0
Phosphorus	P	100.
Sulphate	SO_4	1500.
Sulphide	S	2.0
Tin	Sn	5.0
Zinc	Zn	5.0

Source: City of Toronto By-law 2520, March 4, 1981.

VALUE OF DYNAMIC TESTS IN ACUTE ECOTOXICITY ASSESSMENT IN ALGAE

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FERARD, J.F., P. VASSEUR and J.M. JOUANY. 1982. Value of dynamic tests in acute ecotoxicity assessment in algae. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Sorption of toxic materials by algae should be important and occurs very quickly. Thus, a decrease in the concentration of toxic materials in the experimental medium used for static bioassays results in an under estimation of the toxicity. In this work, two different methods of exposure of Chlorella vulgaris (static and pseudodynamic test) were studied. Acute effects (biological and analytical effects) of inorganic compounds (copper, cadmium, lead and chromium salts) were evaluated for 96-hour tests; in each case the IC$_{50}$ was lower in the dynamic condition than in the static one. The accumulation of metals was calculated for each IC$_{50}$ value and it appears that this parameter is lower when testing by the pseudodynamic method. These results point out the importance of the cinetic factor of toxic distribution during the cell cycle and furthermore the value of pseudodynamic tests.

Key Words: Algae, Chlorella vulgaris, copper, cadmium, lead, chromium, pseudodynamic bioassays, static bioassays.

FERARD, J.F., P.VASSEUR and J.M. JOUANY. 1982. Interet des tests dynamiques algals pour la determination de l'ecotoxicite aigue d'une substance. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'accumulation des toxiques par les algues ne doit pas être négligée d'autant plus que ce phénomène est trés rapide. En effet dans les tests statiques la diminution des concentrations toxiques ajoutées dans le milieu conduit à sous-estimer la toxicité du produit testé. Deux méthodologies (statique et pseudodynamique) ont été étudiées sur une algue Chlorella vulgaris. Les effets aigus de 4 composés inorganiques (cadmium, chrome, cuivre et plomb) ont été envisagés sous l'angle biologique et analytique. La durée d'exposition est de 96 heures. Pour tous les produits testés la CI^{50} est plus faible pour le test pseudodynamique que pour le test statique. Pour chaque CI^{50} obtenue, la quantité de métal accumulé a été calculée et, là aussi, il s'avère que ce paramètre est plus faible dans les conditions pseudodynamiques que dans les conditions statiques. Ces divers résultats soulignent l'importance d'abord des conditions d'apport du toxique en fonction du temps c'est-à-dire durant le cycle cellule, et ensuite l'intérêt des tests pseudodynamiques.

INTRODUCTION

Ecotoxicological testing is aimed at determining undesirable effects of toxicants and at estimating the concentration range of toxicants within which these effects occur. In this respect, "dose-effect" relationships may be estimated by determining the amount of the substance in the test organisms. But more generally the "concentration-effect" relationships are evaluated and effects are expressed by taking into account the level of the toxicant in the test medium, which is referred to as "environmental concentrations". This is done particularly in aquatic ecotoxicological testing. This implies that during the test period, concentrations of the toxicant are controlled in the medium or are maintained constant. This last condition is seldom realized in static aquatic tests because of the absorption of the toxicant of the species being studied. As the concentration factor may be high, toxicant depletion occurs in the test medium and,consequently, yields erroneous results in the determination of the concentration-effect relationship.

Some preliminary trials, relative to the effects of copper on algae, indicated that absorption of this metal by the algal cells began very early, in the first minutes following addition of copper to the medium (Fig.1). Copper depletion in the medium can reach 40% of the initial concentration after 30 minutes of exposure. Such a reduction in the experimental concentrations results in understated readings, at toxicity levels, using this testing procedure. This led us to compare four different methods of testing algae. These were: (1) static test (ST): the toxic compound is added to the medium at the beginning of the test period, the medium is unchanged during the experiment; (2) static test with an adjustment of toxic concentrations (ST-ATC) to repair copper depletion in the medium; (3) dynamic (or continuous flow) test (DT) in which the test medium is supplied at a constant rate to algae with the volume of the suspension remaining constant;(4) pseudo-dynamic test (PDT) which differs from the preceding one in that the volume of the test medium increases with addition of test medium since excess medium is not removed.

First, a preliminary test of the acute effects of copper on *Chlorella vulgaris* was done to evaluate the different results obtained with these four methods. Then the most characteristic ways of exposure were selected and refined studies carried out with copper, cadmium, lead and chronium.

MATERIALS AND METHODS

MATERIALS

The biological material

The algae used was *Chlorella vulgaris* B., strain 211/11b-Chlorphycae obtained from the Algae and Protozoa Center of Cambridge. They are grown inLefevre-Czarda medium (L-C), supplemented with trace elements (AFNOR, 1980). Axenic cultures were produced in 500 ml erlenmeyer flasks fixed on a gyratory shaker at 150 rpm, under 1,500 ± 100 lux and a 16/8 photoperiod. Temperature was 20 ± 1° C (FERARD and MUGEL, 1978). Weekly subcultures were produced by diluting a 7-day culture that contained approximately 30 x 60^6 cells/ml; with fresh LC medium (10% V/V). Algal growth was monitored by measurements of the optical density at 665 nm with a spectrophotometer (Perkin-Elmer-Coleman 285-E) and by counting algal cells using a Coulter Counter (ZB).

The tested compounds

All the experimental compounds used were from PROLABO (Normapur quality):

$Cu\,SO_4,\ 5H_2O$

$Cd\,(NO_3)_2,\ 4\,H_2O$

$Pb\,(NO_3)_2$ (When testing $Pb\,(NO_3)$ the algal culture medium was Lc-medium, without phosphates)

$Cr_2\,O_7K_2$

METHODS

General methodology of the experiment

One ml of a 7-day basic culture, in which the cells had been resuspended in fresh culture medium (LC), after centrifugation and washing, was diluted with 9ml of LC medium, with or without the tested compound, in order to obtain various concentrations of the metal. At the start of the test, the algal content of each tube was the same,about 1.5 to 3 x 10^6 cells/ml. Three replicates of each concentration were tested simultaneously in the growth conditions described in Fig.2: light 2,200 ± 50 lux, agitation 220 rpm. Temperature and photoperiod were the same as for the basic culture.

Several parameters were studied during the test period:
- culture cell number

- opacimetric measurements: transmission percentages of each suspension (T% at 665 nm) are converted to optical density (OD)
- biomass (dry weight determinations)
- metal accumulation by algae and amount left in the supernatent medium.

IC_{50} was determined graphically, taking into account the OD values of the cultures containing toxicant and their corresponding controls.

Detailed methodology of the four different experimental conditions

Static test (ST): Experiments were done in glass test tubes (18/180 mm) set in a support of a gyratory shaker (Fig.3). Twenty tubes can be tested at the same time.

Static test with periodic adjustment of the toxic concentrations (ST-ATC): The apparatus is the same as with the static test. Metal concentrations in the medium were adjusted after 21, 32, and 76 hours of exposure. An aliquot from each different culture was filtered and the amount of toxicant evaluated. Then the toxic compound was added to the tubes, in which a depletion occurred, so as to obtain the initial concentration of toxicant (Fig.4).

Dynamic test (DT): The cell cultures received continuous flow of toxic medium through polyethylene catheters inside diameter 0.30 mm, and a flow rate of 10 ml/24 hours. As the supply of medium has to be delivered equally to each test tube, two capillary siphons in series were used. As indicated in Fig.3, an overflow of the test tubes allowed elimination of excess medium, with constant volume (10 ml) maintained for the complete period of the testing. Agitation insured homogeneity of the algal cultures, but may also result in a decreasing number of chlorellae cells in the test medium, because a fraction of the algal biomass was eliminated with the excess suspension.

Pseudo-dynamic test (PDT): The methodology differs from the dynamic one in that test tubes are not provided with an overflow. So the volume of the algal suspension increased as toxic medium was added. For this test, large test tubes were used (24 x 220 mm) to insure adequate capacity. Moreover as it was difficult to provide an equal flow rate of medium to each tube during the test, so medium was added to the tubes periodically. Thus, the fresh medium was delivered manually with an automatic pipette, five timer per day (1 ml in each tube every three hours: at 7, 10, 13, 16, and 19h). This technique was easier to use than the dynamic test, since the whole algal biomass was kept for analysis and each tube contained the same volume of culture which facilitated comparison of OD values.

Analytical metal determination

An aliquot of an algal culture was filtered through a Millipore membrane (0.45 u) and washed twice with double distilled water. The filter was mineralized according to the technique of Boudene et al. (1976). Metal determinations were done by atomic absorption spectrophotometer using a Perking-Elmer 306 B, fit out with an HGA-76 furnace and an automatic injection system. The analytical conditions are summarized in Table 1.

RESULTS

COMPARISON OF THE FOUR DIFFERENT TESTS WITH COPPER

The various concentrations of copper tested using the four different techniques previously mentioned were: 0, 50, 100, 150, 200, 250, 300, 350, 500 ug/l of algal suspension. Determination of the IC_{50} values for 72 and 96 hours of exposure are given in Table 2. Toxicity of copper appears to be greatest in the dynamic conditions, when the toxic medium is introduced during the test. Toxicity is respectively two and four times higher in the dynamic and pseudodynamic tests than in the station one, which gives the most optimistic result. No significant difference can be detected between 72 and 96 h of exposure. The amount of copper in the algae was measured. Table 3 shows that accumulation increased when copper was supplied during the test period. We define the concentration factor (FC) as follows:

$$FC \quad \frac{\text{Concentration of Cd in algae at } t = 96 \text{ h} \\ \text{(in mg/kg dry weight)}}{\text{Concentration of Cd added in medium at } t\text{=}0 \\ \text{(in mg/l)}}$$

Fig. 5 shows that the concentration factor is nearly three times higher in the pseudodynamic test than in the static test and increases tenfold when copper concentration is adjusted (in the medium). It can be noticed that FC is correlated with the metal concentration in the medium. No analytical determination could be done with the dynamic test, since the biomass was much too small. Therefore we did not select this method of exposure of the Chlorellae in the main study, nor did we use the test with an adjustment of toxic concentration, which is never carried out in algal ecotoxicity testing because of the too time consuming and expensive methodology that it implies. Nevertheless, it was interesting in a preliminary investigation to compare these two methods of exposure with the pseudodynamic one. The pseudodynamic test requires no specific apparatus, and no difficult procedures. Even if the increase in toxicity that it reflects is not so high as the true dynamic test, the difference in results compared to the static conditions is important enough to be pointed out.

COMPARISON OF THE STATIC AND PSEUDO-DYNAMIC TEST WITH COPPER, CADMIUM, LEAD AND CHROMIUM

In the following trials, the acute reflects of the four tested metals have been compared using the static and pseudo-dynamic methods of exposure of the Chlorellae. Each compound was tested four times (n = 4). Biological effects were evaluated after 72 and 06 h (Table 4).

Results are the same as previously; in every case IC_{50} values are lower in the pseudo-dynamic test than in the static one. After 96 h the percentages of decrease defined as:

$$\frac{IC_{50}\ ST - IC_{50}\ PDT}{IC_{50}\ ST} \times 100$$

are respectively 58, 55, 74, and 73% for copper, cadmium, lead, and chromium respectively. The decreases in IC_{50} are nearly the same after 72 h. of exposure. So, as in the preliminary assays, there is no significant difference in toxicity between these two periods of exposure. The amounts of toxicant accumulated in the algae and remaining in the supernatent fluid were evaluated at the end of each assay. The average results for the four tested metals are given in Tables 5, 6, 7, and 8. The concentrations of toxicant left in the supernatents after 96 h are very low for every compound in both the pseudo-dynamic and static tests. In the case of copper (Table 5) the concentration in the supernatent was lower in the pseudo-dynamic test (medium value = 37%) than in the static method of exposure (60%). This may be correlated with the higher accumulation of metal by algae in the pseudo-dynamic test. The concentration factors in the pseudo-dynamic test was about three times higher than in the static test, as in the preliminary assay (Table 5, Fig.6). Results are different with cadmium (Table 6, Fig.6) where the levels founa in Chlorellae are of the same order of magnitude in the two conditions. The mean value of CF was nearly 4.000 in both cases. On the contrary, the concentrations of this metal in the static supernatent were small (average percentage = 19%/static and 32%/pseudo-dynamic). The same remark can be made for lead (8%/static and 12%/pseudo-dynamic). In this last test the quantities of lead found in algae are at least twice as high in the pseudo-dynamic exposure. The mean values of the concentration factor corroborate this (Table 7, Fig.6). As for chronium, no significant difference was found at the same concentrations of this toxic in the medium with these two methods of exposure. CF did not exceed 1.000 in any assay (Table 8).

DISCUSSION - CONCLUSION

When looking at the results of the static tests, it appears that biological effects of the experimental compounds are similar to the results found in other works. Thus IC 50-96 h values are of the same order of magnitude as the results found for copper (Young and Lisk 1972; Gea 1980) cadmium (Ferard and Mugel 1978; Truhaut et al. 1980) lead (Kee and Wolg 1963) and chromium (Ircha, 1981; Vasseur et al. 1982). No bibliographic data exist for dynamic conditions. The lower IC_{50} values in the dynamic tests show that Chlorellae are more strongly affected with such an exposure. This increase of toxicity may be correlated with an higher concentration factor of metal, for copper and lead. We considered the dose-effect relationship to determine the amounts of metal really accumulated in algae which were responsible for the 50% decrease of algal growth. Thus the levels of toxicant in these organisms have been calculated, for an exposure corresponding to each IC 50-96 h concentration. It appears (Table 9) that the same biological effects occur for an accumulation of toxicant of a lower concentration in the pseudodynamic exposure than in the static one. Except for copper, the amount of metal accumulated is nearly twice as low in the pseudo-dynamic as in the static test. Such results mean that parameters other than the absolute level of toxicant in algae is responsible for the observed toxicity. It may be the kinetics of distribution of the toxic substance which is delivered continuously throughout the cell cycle in the dynamic test. Sensitivity to some toxics is influenced by the state of the cells, in particular the phases of their cycles (WRIGHT, 1981). As in the aquatic ecosystem, algae are exposed to toxicants under dynamic conditions in the test, hence it would be fair in ecotoxicity assessment to take into account this important parameter because this series of experiments shows that static tests give optimistic results and understate toxicity. Dynamic tests are difficult to carry out and an important fraction of the algal biomass is last with the overflow. This implies regular and identical discharge of medium from every flask, if the OD criterion is to used to evaluate algal growth. This fact may explain, first, why algal tests are generally done with static exposure and, second, that toxicity to fish is usually evaluated with dynamic tests where this kind of problem does not occur (Pickering and Gast 1972; I.S.O., 1980; O.E.C.D., 1981). In this context, it would be best to choose a pseudo-dynamic method of exposure, which is very easy to use for representative assessment of toxicity in algae.

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TABLE 1 : OPERATING CONDITIONS FOR FLAMELESS ATOMIC ABSORPTION SPECTROPHOTOMETER DETERMINATIONS OF METALS

	COPPER	CADMIUM	LEAD	CHROMIUM
WAVELENGTH (NM)	324.7	228.8	283.3	358
SLIT SETTING (NM)	0.7	0.7	0.7	0.7
ARGON PRESSURE (BARS)	3	3	2.2	2.2
PRIMARY PHASE (DRYING)	105°C 30 SEC.	100°C 20 SEC.	100°C 20 SEC.	100°C 30 SEC.
SECONDARY PHASE (MATRIX DESTR.)	350°C 20 SEC.	700°C 15 SEC.	700°C 15 SEC.	1350°C 20 SEC.
TERTIARY PHASE (ATOMIZATION)	1800°C 10 SEC.	2450°C 5 SEC.	2450°C 5 SEC.	2650°C 5 SEC.

TABLE 2 : IC50 VALUES OF COPPER AFTER A 72 AND 96 HOURS GROWING PERIOD OF CHLORELLA CULTURES IN VARIOUS CONDITIONS OF EXPOSURE (EXPRESSED IN UG/L)

EXPOSURE TIME (HOURS) / TEST	72	96
STATIC	240	265
STATIC WITH ADJUSTMENT OF TOXIC CONCENTRATIONS	85	105
DYNAMIC	65	62
PSEUDO-DYNAMIC	164	164

TABLE 3 : DISTRIBUTION OF COPPER IN CHLORELLAE CULTURES AFTER A 96 HOURS GROWING PERIOD IN DIFFERENT CONDITIONS

INITIAL COPPER CONCENTRATION IN uG/L		50	100	150	200	300	500
COPPER CONCENTRATION IN SUPERNATENT (IN uG/L)	ST	35	40	72	70	47	72
	PDT	18	22	26	37	46	86
	ST-ATC	46	99	112	138	190	305
COPPER CONCENTRATION IN ALGAE (MG/KG DRY WEIGHT)	ST	40	110	173	315	750	3,200
	PDT	80	296	600	829	1,273	3,000
	ST-ATC	245	584	1,711	3,240	3,750	4,300
CONCENTRATION FACTOR	ST	800	1,100	1,150	1,575	2,500	6,400
	PDT	1,600	2,960	4,000	4,145	4,143	6,000
	ST-ATC	4,900	5,840	11,400	16,200	12,500	8,600

ST = STATIC TEST
PDT = PSEUDODYNAMIC TEST
ST-ATC = STATIC TEST WITH ADJUSTMENT OF TOXIC CONCENTRATION

TABLE 4 : COMPARISON OF IC_{50} VALUES (MG/L) IN STATIC AND PSEUDODYNAMIC CONDITIONS FOR DIFFERENT MINERAL COMPOUNDS AND TWO TIMES OF EXPOSURE (N = 4)

COMPOUND TEST		COPPER	CADMIUM	LEAD	CHROMIUM
STATIC	72 H	0.23 ± 0.08	1.21 ± 0.20	1.83 ± 0.21	0.47 ± 0.16
	96 H	0.27 ± 0.07	1.22 ± 0.19	2.20 ± 0.31	0.59 ± 0.26
PSEUDODYNAMIC	72 H	0.10 ± 0.06	0.65 ± 0.09	0.53 ± 0.20	0.12 ± 0.01
	96 H	0.11 ± 0.06	0.55 ± 0.05	0.57 ± 0.26	0.16 ± 0.01
% REDUCTION OF IC_{50}	72 H	57 %	46 %	71 %	74 %
	96 H	58 %	55 %	74 %	73 %

TABLE 5 : DISTRIBUTION OF COPPER IN CHLORELLAE CULTURES AFTER A 96 HOURS GROWING PERIOD IN STATIC AND PSEUDODYNAMIC CONDITIONS (N = 4)

COPPER CONCENTRATION ADDED IN MEDIUM (IN uG/L)		50	75	100	150	200	250	300
COPPER CONCENTRATION IN SUPERNATENT IN uG/L)	ST	57 ± 12	*	85 ± 21	116 ± 27	120 ± 17	153 ± 43	188 ± 3
	PDT	43 ± 16	45 ± 6	67 ± 34	71 ± 19	96 ± 25	**	**
COPPER CONCENTRATION IN ALGAE (IN MG/KG DRY WEIGHT)	ST	119 ± 45	*	265 ± 120	482 ± 257	763 ± 300	958 ± 460	1,011 ± 392
	PDT	161 ± 48	387 ± 140	955 ± 320	1,386 ± 885	1,545 ± 700	**	**
CONCENTRATION FACTOR	ST	2,380	*	2,650	3,210	3,815	3,830	3,370
	PDT	3,220	5,160	9,550	9,240	7,725	**	**

* = NO DATA : CONCENTRATION NOT TESTED

** = NO DATA : CONCENTRATION NOT TESTED, FOR IT IS TOO MUCH TOXIC IN PSEUDO DYNAMIC CONDITIONS

TABLE 6 : DISTRIBUTION OF CADMIUM IN CHLORELLAE CULTURES AFTER A 96 HOURS GROWING PERIOD IN STATIC AND PSEUDODYNAMIC CONDITIONS (N = 4)

CADMIUM CONCENTRATION [illegible] MEDIUM (IN µG/L)		300	500	1000	1500	2,000	2,500	3,000
CADMIUM CONCENTRATION IN SUPERNATENT (IN µG/L)	ST	34 ± 20	72 ± 34	202 ± 33	346 ± 46	594 ± 137	1,168 ± 168	1,515 ± 342
	PDT	59 ± 11	116 ± 16	294 ± 30	564 ± 298	1,237 ± 325	**	**
CADMIUM CONCENTRATION IN ALGAE (IN MG/KG DRY WEIGHT)	ST	1,338 ± 383	2,021 ± 940	4,427 ± 554	6,445 ± 1,910	5,780 ± 770	9,700 ± 2,220	12,820 ± 3,684
	PDT	1,224 ± 390	2,585 ± 1,087	4,552 ± 2,049	7,405 ± 4,339	**	**	**
CONCENTRATION FACTOR	ST	4,460	4,042	4,427	4,300	2,890	3,880	4,273
	PDT	4,080	5,170	4,550	4,930	**	**	**

** = NO DATA : CONCENTRATION NOT TESTED, FOR IT IS TOO MUCH TOXIC IN PSEUDO DYNAMIC CONDITIONS

TABLE 7 : DISTRIBUTION OF LEAD IN CHLORELLAE CULTURES AFTER A 96 HOURS GROWING PERIOD IN STATIC AND PSEUDODYNAMIC CONDITIONS (N = 4)

LEAD CONCENTRATION ADDED IN MEDIUM (UG/L)		500	750	1,000	1,500	2,000	2,500	3,000
LEAD CONCENTRATION ADDED IN MEDIUM (UG/L)	ST	46 ± 5	*	49 ± 23	98 ± 15	101 ± 8	217 ± 140	402 ± 330
	PDT	50 ± 15	62 ± 15	114 ± 51	122 ± 30	196 ± 20	*	390 ± 38
LEAD CONCENTRATION IN ALGAE (MG/KG DRY WEIGHT)	ST	771 ± 870	*	1,670 ± 1,090	3,050 ± 1,580	5,680 ± 4,300	6,300 ± 4,160	8,345 ± 4,710
	PDT	1,610 ± 520	2,180 ± 805	3,764 ± 2,300	7,765 ± 2,500	12,516 ± 8,210	*	13,263 ± 5,420
CONCENTRATION FACTOR	ST	1,540	*	1,670	2,035	2,840	2,520	2,780
	PDT	3,300	2,906	3,760	5,175	6,260	*	4,420

* = NO DATA : CONCENTRATION NOT TESTED

TABLE 8 : DISTRIBUTION OF CHROMIUM IN CHLORELLAE CULTURES AFTER A 96 HOURS GROWING PERIOD IN STATIC AND PSEUDODYNAMIC CONDITIONS (N = 4)

CHROMIUM CONCENTRATION ADDED IN MEDIUM (UG/L)		100	200	400	600	800	900
CHROMIUM CONCENTRATION IN SUPERNATENT (UG/L)	ST	64 ± 6	139 ± 1	304 ± 17	525 ± 19	675 (1)	691 ± 110
	PDT	71 ± 7	162 ± 1	356 ± 16	551 (1)	**	**
CHROMIUM CONCENTRATION IN ALGAE (MG/KG DRY WEIGHT)	ST	84 ± 5	158 ± 15	245 ± 56	370 ± 101	765 ± 156	890 ± 240
	PDT	75 ± 5	151 ± 17	366 ± 114	415 ± 90	**	**
CONCENTRATION FACTOR	ST	840	790	612	616	956	988
	PDT	750	755	915	692	**	**

** = NO DATA ; CONCENTRATION NOT TESTED FOR IT IS TOO MUCH TOXIC IN PSEUDO DYNAMIC CONDITIONS

(1) = WITHOUT STANDARD ERROR BECAUSE N = 2

TABLE 9 : EVALUATION OF CUMULATED METAL IN ALGAE
A 96 HOURS-PERIOD OF EXPOSURE TO THE IC_{50} CONCENTRATION

		Compounds tested			
		Copper	Cadmium	Lead	Chromium
$IC_{50-96\ H}$ (mg/l)	ST	0.27	1.22	2.20	0.59
	PDT	0.11	0.55	0.57	0.16
Concentration in algae (mg/kg dry weight)	ST	1,030	5,000	5,450	484
	PDT	772	2,522	1,935	130

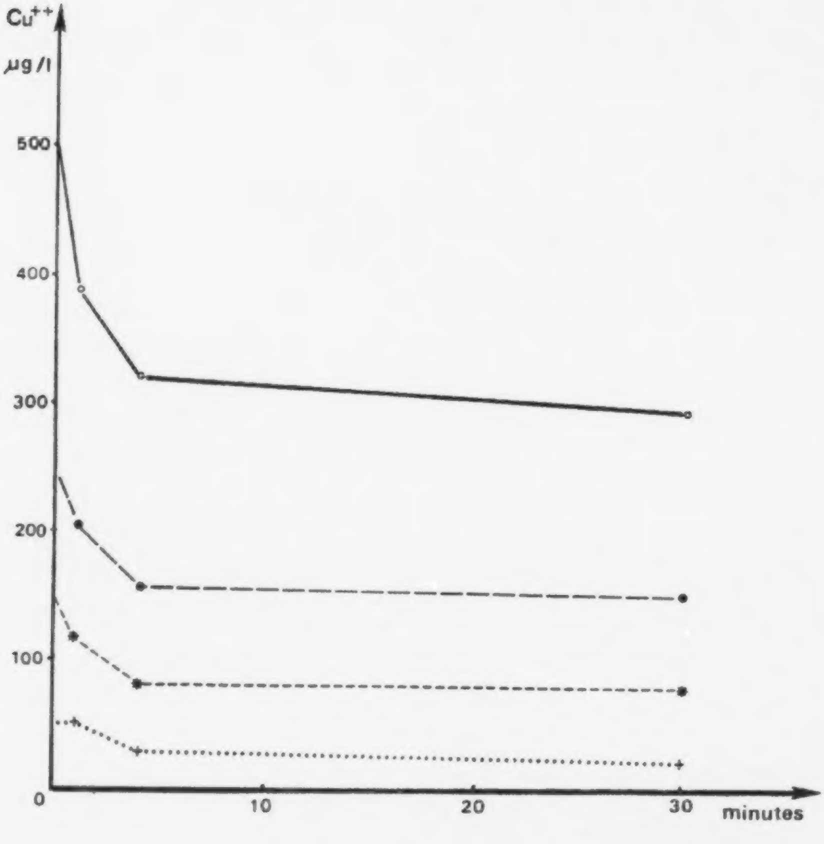

FIGURE 1 : KINETICS OF DECREASE OF COPPER CONCENTRATIONS IN SUPERNATENTS OF CHLORELLAE SUSPENSIONS (1.3 X10^5 CELLS/ML) EXPOSED TO DIFFERENT COPPER CONCENTRATIONS FOR 1,4 AND 30 MINUTES.

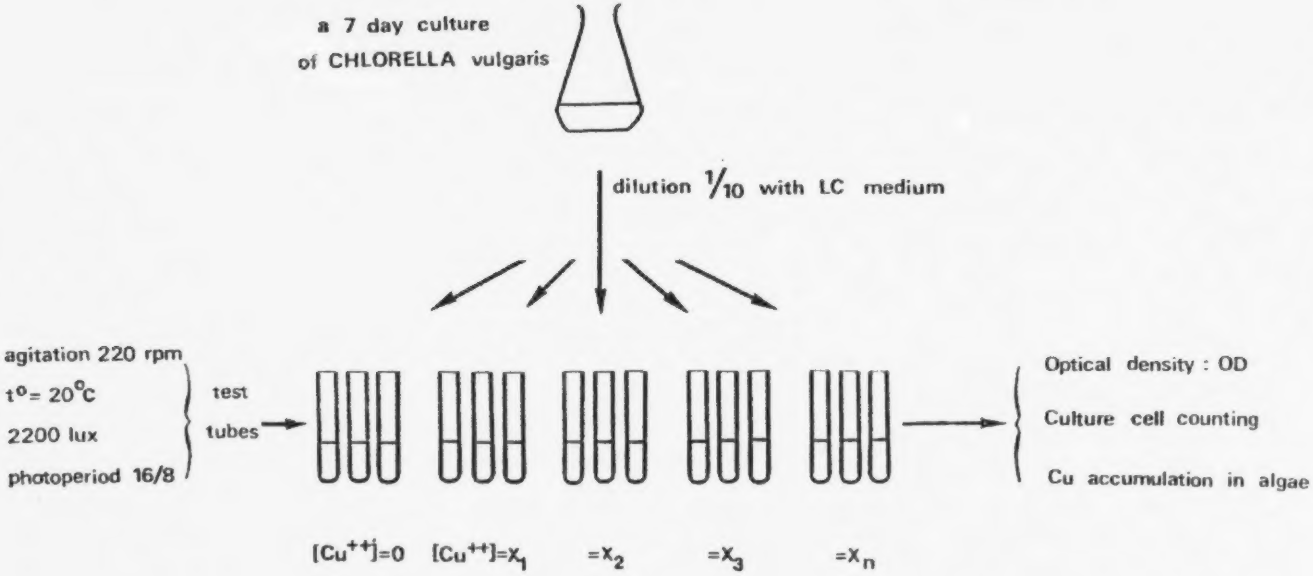
a 7 day culture
of CHLORELLA vulgaris
dilution 1/10 with LC medium
agitation 220 rpm
t° = 20°C
2200 lux
photoperiod 16/8
test
tubes
Optical density : OD
Culture cell counting
Cu accumulation in algae
[Cu++]=0
[Cu++]=x1
=x2
=x3
=xn

FIGURE 2 : SCHEMATIC METHODOLOGY OF THE EXPERIMENTATION

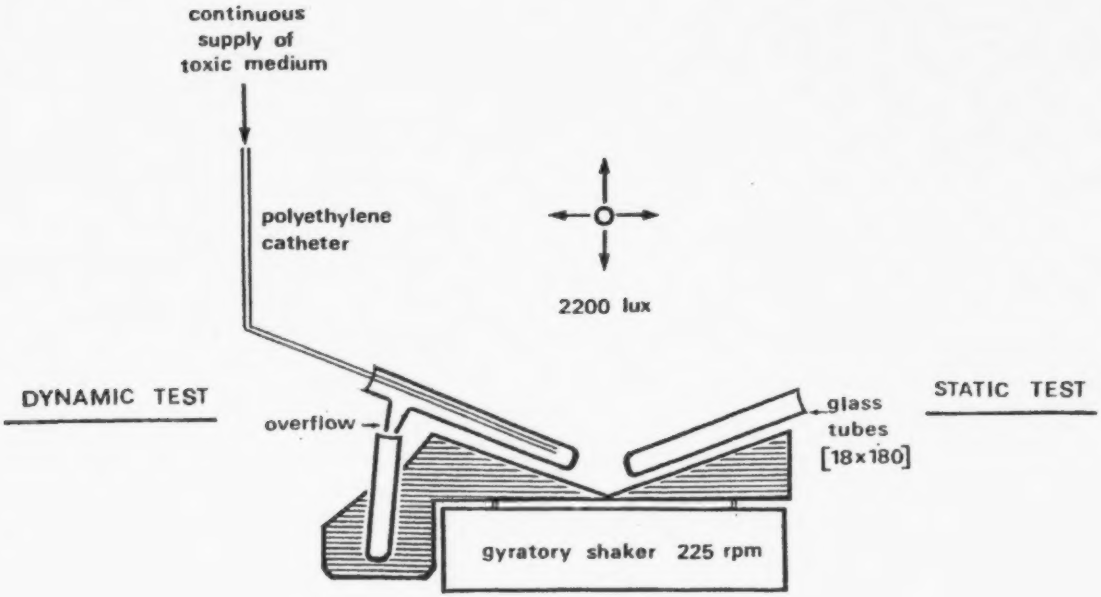
continuous supply of toxic medium
polyethylene catheter
2200 lux
DYNAMIC TEST
overflow
STATIC TEST
glass tubes [18×180]
gyratory shaker 225 rpm

FIGURE 3 : SCHEMA OF THE STATIC AND DYNAMIC TESTS

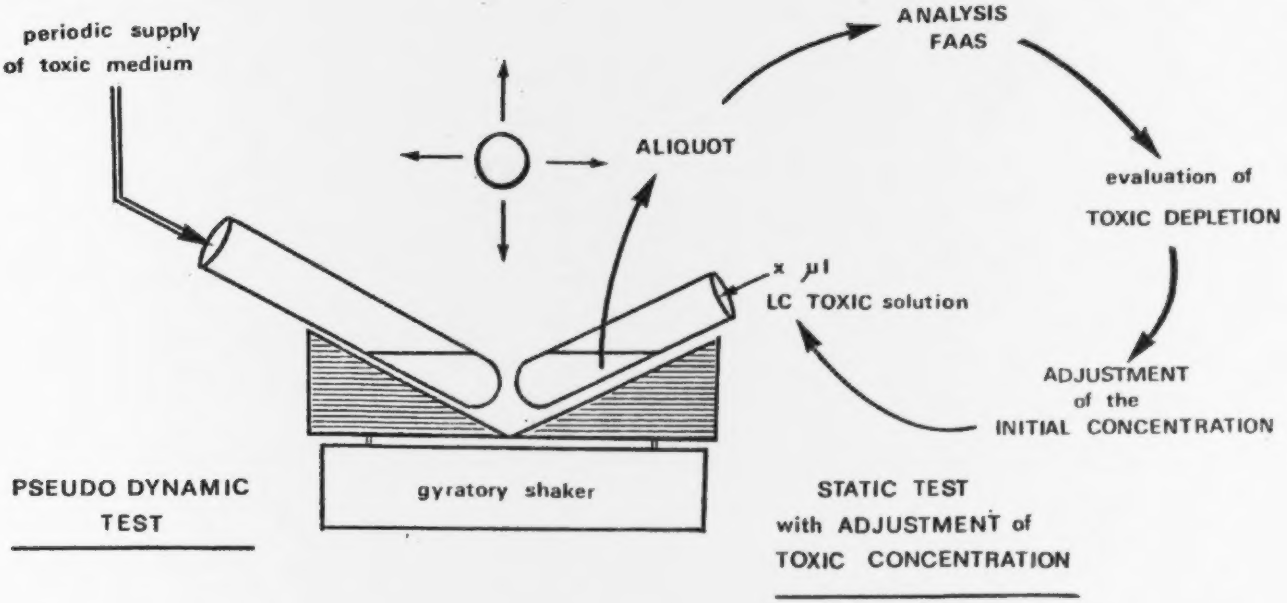
periodic supply
of toxic medium
ALIQUOT
ANALYSIS
FAAS
evaluation of
TOXIC DEPLETION
x µl
LC TOXIC solution
ADJUSTMENT
of the
INITIAL CONCENTRATION
gyratory shaker
PSEUDO DYNAMIC
TEST
STATIC TEST
with ADJUSTMENT of
TOXIC CONCENTRATION

FIGURE 4 : SCHEMA OF THE APPARATUS OF THE PSEUDO-DYNAMIC TEST AND THE STATIC TEST WITH ADJUSTMENT OF TOXIC CONCENTRATION

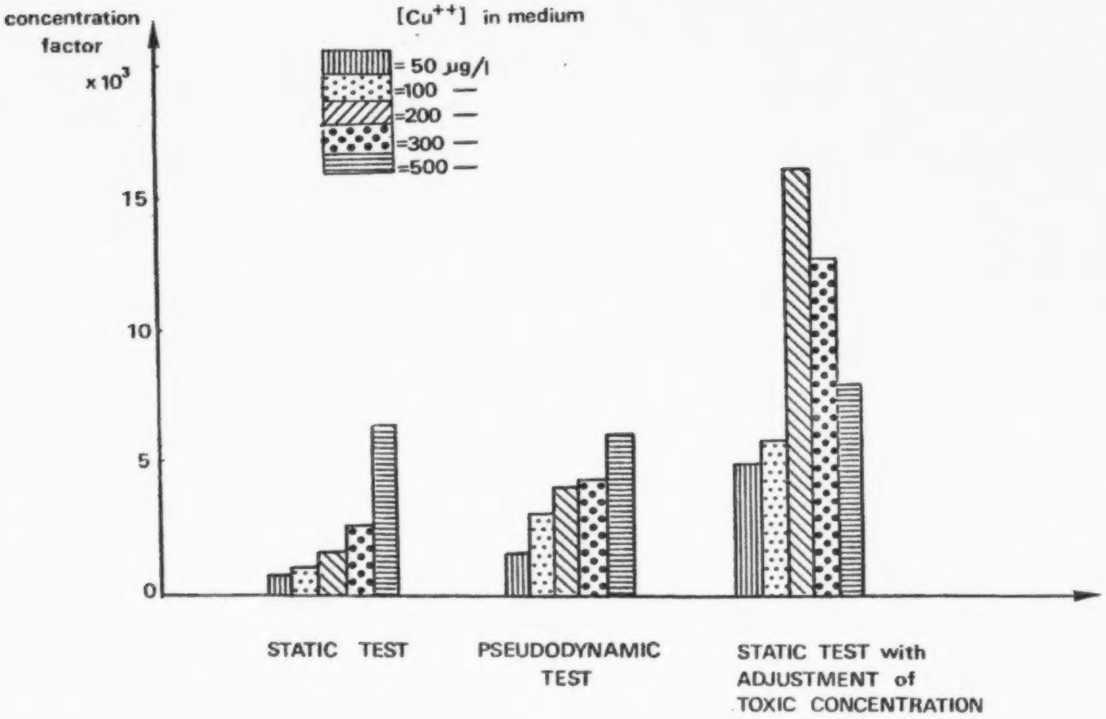

FIGURE 5 : CONCENTRATION FACTOR OF COPPER IN CHLORELLAE

FOR THREE WAYS OF EXPOSURE (T=96 HS.)

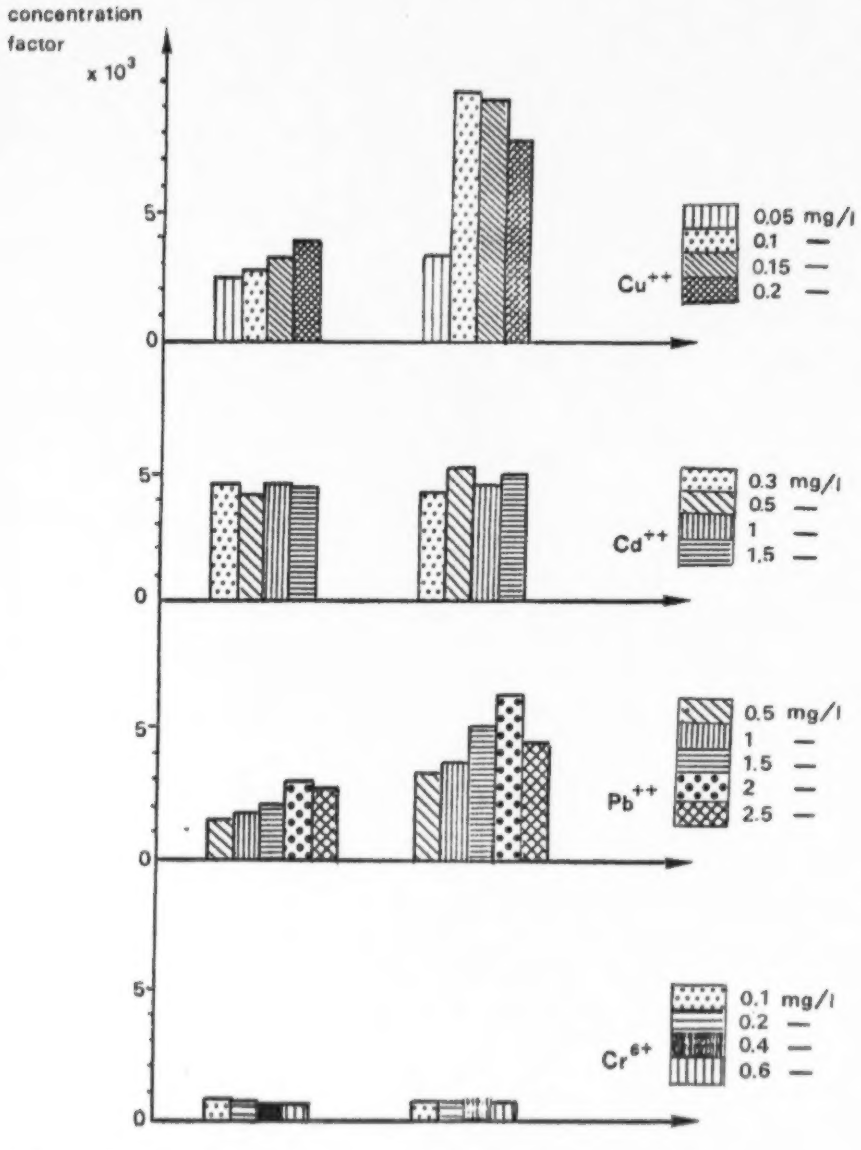

FIGURE 6 : CONCENTRATION FACTOR OF SOME INORGANIC COMPOUNDS IN CHLORELLAE AFTER 96 HOURS OF EXPOSURE IN STATIC OR PSEUDO-DYNAMIC CONDITIONS

ASSESSING INDUSTRIAL WASTEWATER WITH A CONTINUOUS AND AUTOMATED ON-LINE TOXICITY MONITORING SYSTEM

David Gruber and W. Roscoe Miller III

Biological Monitoring Inc., Blacksburg, Virginia U.S.A.

GRUBER, David and W. Roscoe MILLER III. 1982. Assessing industrial wastewater with a continuous and automated on-line toxicity monitoring system. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

A unique on-line Biological Monitoring Facility (BMF) has been developed to automatically and continuously monitor complex effluents at a U.S. Army Ammunition Plant. The BMF assesses wastewater quality by monitoring the ventilatory behavior of fish and several physiochemical parameters, with an on-line minicomputer system. This BMF is housed within a 10 x 4 m trailer. If a number of fish simultaneously breathe abnormally, or if the physiochemical parameters drift outside of the user-inputted acceptable range, an alarm routine automatically generates both audible and visual signals and then places several telephone calls to notify the proper personnel of an alarm condition. Biological alarms trigger within 20 minutes after the introduction of a diluted effluent.

Key Words: Biological Monitoring Facility, wastewater quality, fish, ventilatory rate.

GRUBER, David and W. Roscoe MILLER III. 1982. Évaluation des eaux résiduaires industrielles à l'aide d'un système en direct de surveillance continue de la toxicité. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Une installation unique de surveillance biologique en direct est mise au point en vue de permettre le contrôle automatique et continu des effluents complexes d'une usine de munitions des Forces armées américaines. L'installation analyse la qualité des eaux résiduaires d'après le rythme respiratoire de poissons et divers paramètres biochimiques, grâce à un mini-ordinateur fonctionnant en direct. Elle est logée dans une remorque de 10 m sur 4 m. Dès qu'un certain nombre de poissons respirent de facon anormale ou que les paramètres biochimiques débordent la plage acceptable fixée par l'utilisateur, un programme d'alerte actionne automatiquement des signaux sonores et visuels et établit des communications téléphoniques pour en aviser le personnel compétent. L'alerte est déclenchée dans les vingt minutes qui suivent l'introduction d'un effluent dilué.

INTRODUCTION

The feasibility of using fish ventilatory rates to assess toxicity has been well documented, and numerous laboratory studies have illustrated the sensitivity of fish ventilatory behavior to a variety of compounds (1-38). The field verification of the use of fish breathing patterns as a biological monitoring tool has been scantly researched as few systems have been transferred from the laboratory to an industrial setting. Research by van der Schalie (39) reported on a fish ventilatory monitoring station at an industrial site. This first generation biomonitoring system did not verify the field application of this approach.

Research by Gruber et al. (7-10) on a second generation biomonitoring system has been presented. Recently this system was interfaced to a 1.25 MGD rotating biological contactor (RBC) plant designed to treat a complex industrial effluent.

The purpose of this study was to examine the relationships between acute toxicity (96-hr LC50) and the ventilatory response of fish sensors. These data were then used to present a case for the application of a Biological Monitoring Facility (BMF) as the primary means of on-line monitoring of toxic substances.

MATERIALS AND METHODS

The BMF was interfaced to the RBC plant such that wastewater could be collected from three points in the treatment system. The first sampling point was a pretreated influent to an equalization basin. The second sampling point was the influent to the RBC units. The third sampling point was the final effluent to the receiving system.

Principal components of the BMF were a diluter system, ventilatory tanks and module, electronics to amplify the ventilatory signal, and a minicomputer system used to count, store and assess the ventilatory rates of twelve bluegill (Lepomis macrochirus). Other components of the system included: a bioassay diluter, an automatic water sampler, and a water quality analyzer (interfaced to the minicomputer) for monitoring DO, temperature, pH, and conductivity. These components have been more thoroughly described elsewhere (7-10).

Bluegill used in the ventilatory system were obtained from a commercial hatchery and were 6-10 cm. Bluegill used in the acute bioassay diluter were 2.5 to 3.8 cm. All organisms were maintained under flow-through conditions using receiving system dilution water obtained upstream from the plant.

An experiment was started by first introducing wastewater, from one of the three sampling points, into the bioassay diluter. This was done at least 24-hr prior to the introduction of the organisms. Before introducing the organisms, DO, temperature, and pH were recorded and subsequently at 24-hr intervals for the remainder of the 96-hr experimental period. At the beginning of a bioassay, 10 randomly selected bluegill were introduced into each of twelve containers. A logarithmic dilution series of the wastewater was used. At each concentration there was one replicate. Ninety-six-hour LC50 values were calculated using probit and Spearman-Karber techniques (40,41).

Ventilatory rate data were recorded from twelve bluegill. During wastewater exposure four bluegill were exposed exclusively to diluent (i.e., upstream dilution water). The remaining eight bluegill were exposed to a metered dilution of the wastewater. Ventilatory rate data were collected in ten-minute intervals. A sequential moving average approach was used to determine if any of the twelve bluegill significantly altered its ventilatory rate over time. A computer algorithm provided a user option for determining how many fish had to begin breathing abnormally before an alarm was triggered.

Basically, two levels of alarms were provided. One, when a user-optioned number of wastewater-exposed fish began breathing abnormally. The second alarm was triggered when a combination of wastewater exposed and diluent-exposed fish began breathing abnormally. These alarms were classified into a high and a low priority alarm, respectively.

RESULTS

Data from six 96-hr bioassays and simultaneous ventilatory rate experiments are provided in Table 1. The 96-hr LC50's ranged from a low of 29% (most toxic) to 58% (least toxic). The frequency of high priority alarms prior to effluent introduction (a false alarm) was quite low. From a total of 39,910 minutes of pre- and post-exposure ventilatory monitoring, one high priority alarm was recorded (i.e., a false alarm rate of 0.025%). During four (A,C,D, and E) of the six experiments the animals in the ventilatory apparatus were exposed to a dilution of the wastewater which was 1.4 to 1.7 times the 96-hr LC50. During the remaining two (B and F) experiments the animals were exposed to 0.52 (B) and 0.86 (F) of the 96-hr LC50.

The number of high and low priority alarms along with the time (minutes) to alarm is also provided in Table 1. The variables which appeared to best correlate with the observed 96-hr LC50 sequence was the time to the first high priority alarm. The LC50 data ordered from most toxic to least toxic were - E>C>D>F>A>B. Based on time to first high priority alarm the sequence was E>D>C>A>F>B. Considering the small differences in LC50's between experiments A,C,D and F these data correlate quite well.

An illustration of the types of data collected from a ventilatory experiment is presented in Figures 1 to 3 for Experiment A. Data for four (4) control fish(0 to 3) and eight (8) wastewater exposed fish (4 to 11) are presented. The wastewater was introduced beginning at hour 19.3 and turned off at hour 21. At least five aspects of fish ventilatory behavior are evident from these data. One, fish breathe at different rates. All fish were held under identical conditions and all were of the same age class. However, some fish breathe at a relatively constant rate (e.g., fish 0) while others are more erratic (e.g., fish 2). Two, warnings (individual statistical departure from a previously user-optioned set of variables) were generated by control animals and during pre-exposure conditions. Three, the typical response to wastewater exposure was a simultaneous decrease in ventilatory frequency coupled with an increase in the variability of the ventilatory data. Four, warnings occurring prior to effluent introduction were dealt with via the critical limits recalculation subroutine, and did not contribute to a false alarm. Five, the number of warnings for this experiment increased 2.4 times following effluent introduction.

An analysis of the data from all six experiments revealed what appeared to be a logarithmic relationship between the time to high priority alarm and what percentage of the LC50 the ventilatory monitors were exposed to. Using the logarithmic model

$$t = \alpha e^{\beta \frac{LC50}{conc}}$$

where:
t = time to high priority alarm
LC50/conc = 96 hr LC50/ventilatory exposure concentration
e = base of the natural logarithms
α, β = constants

a linear regression was performed on the log transformed data points, yielding:

$$t = 0.67e^{4.42 \frac{LC50}{conc}}$$

The relationship had a correlation coefficient of 0.95; thus, the model was assumed to apply. The results are shown in Figure 4.

DISCUSSION

The major thrust of these experiments was the field verification of the BMF. Even though the 96-hr LC50 fluctuated from 29% to 58% wastewater, the ventilatory system responded quickly to toxic thresholds at 0.52 to 1.72 times the 96-hr LC50. These data have indicated that a BMF could be used to continuously monitor effluent toxicity.

The present BMF has or will incorporate several other features which should enhance its field utility. One, an automatic water sampler is currently interfaced to the computer such that a wastewater sample is automatically collected upon alarm generation. Consequently, a wastewater sample is provided at the time a toxicity threshold is exceeded. Subsequent analyses of this sample could help identify process points causing the observed toxic response and suggest possible treatment activities necessary to reduce effluent toxicity. Second, an automated telephone message generating system will be interfaced to the computer. This will enable the communication of an alarm condition to plant personnel. In this manner, plant personnel can check the computer for the alarm priority, pick up the automatically collected wastewater sample, and initiate the required analysis. Third, the alarm signal could be interfaced to an electronic valve such that the final effluent could be diverted to a holding pond or recycled to the headworks of the treatment system. These events have been summarized in Figure 5.

Notice that if the effluent is approved for discharge, no permit (NPDES) testing would be warranted. However, several conditions should be met prior to pursuing this course of action. One, the ventilatory system must be calibrated on-site to acute bioassay results. Two, the plant's treatment must be of sufficient reliability such that the treatment of conventional pollutants are of secondary importance to the toxics present. Three, well trained personnel must be available to make decisions based on the BMF's output (i.e., divert the wastewater, additional treatment needed, recycle the wastewater, etc.).

An important question in the ultimate functioning of biomonitoring systems at industrial sites is - what dilution of effluent should the sensors be exposed to? It is unreasonable to expect that 100% effluent would not illicit a ventilatory response. It is also unreasonable to expose the sensors to the previously determined 96-hr LC50 since this may also present the sensors with an unrealistically high exposure. It could be suggested that the sensors be exposed to the in-stream waste concentration (IWC) as determined by the equation:

$$IWC\ (\%) = \frac{Qw}{Qr + Qw} \times 100$$

where:
Qw = effluent flow (MGD)
Qr = 7Q10 (7 day, 10 year low flow of receiving system in MGD)

For example, the plant that the BMF is currently interfaced to has a final effluent flow (Qw) = 1.5 MGD. The 7Q10 (as determined by USGS records) at a point several miles above the plant is 43.3 MGD. Consequently, the IWC by calculation equals 3.35 percent. Appropriate application factors can be incorporated into this figure which could partially account for differences between persistent and non-persistent pollutants and toxicity in and around a mixing zone.

Alternatively, a relationship similar to that depicted in Figure 4 could be used to predict the exposure concentration. The selection of the exposure concentration could be based on a 96-hr LC50 and the sensitivity (time to alarm) desired.

WHY BIOLOGICAL VERSUS PHYSIOCHEMICAL MONITORING?

The technologies of physiochemical monitoring have experienced tremendous growth and sophistication over the past three decades. As a consequence, chemical and physical monitors have traditionally been used for assessing water quality. However, there are seven distinct disadvantages to this approach. One, monitoring physiochemical characteristics cannot be used to directly determine toxicity. Two, chemical and physical monitors cannot determine synergistic, additive, or antagonistic effects. Three, physiochemical monitors are also very specific, monitoring only a small percentage of potentially harmful substances. Four, chemical substances may have adverse biological effects at concentrations below present analytic capabilities. Five, few systems can be used continuously and automatically and most chemical analyses incorporate expensive, time consuming and elaborate technologies requiring skilled technicians. Six, many of the more elaborate analytical techniques are subject to interferences making data interpretation difficult. Seven, the time to collect, analyze, and report physiochemical test results often preclude prompt pollution abatement action.

In contrast to the physiochemical approach, biological monitoring systems utilize a living sensor to integrate the myriad biological, chemical, and physical factors which taken together determine toxicity. The fish ventilatory system presented here has been shown to be directly correlated to the 96-hr LC50, a value that to a large extent determines effluent and stream standards. Consequently, this system provides a direct means of continuously and automatically monitoring effluent or in-stream toxicity.

VALUES AND LIMITATIONS OF AUTOMATED BIOMONITORING

Wastewater dischargers have effluent limitations (e.g., kg day^{-1} of COD) which require that wastewater samples be collected at regular intervals (typically once day^{-1} or once week^{-1}). At many facilities, expenditures for these requirements make up a significant portion of the funds marked for multimedia environmental control. To reduce these expenditures, a continuous and automated biological monitoring system could be used to dictate the frequency of required wastewater sampling for chemical monitoring, thus reflecting substantial cost savings for monitoring requirements.

The Office of Water Enforcement of the U.S. EPA has formally indicated that a fish ventilatory system, such as the one described in this paper, may be used to determine when wastewater samples should be collected and analyzed. Upon generation of a priority alarm, the BMF automatically collects wastewater samples and notifies the proper plant personnel. The collected effluent samples could then be analyzed, the constituents(s) causing the alarm identified, and steps initiated to locate the problem area(s) within the production or treatment process. Once problem areas are located and corrective measures taken the frequency of subsequent alarms would decrease, thereby reducing the number of chemical analyses required. Since the operation and maintenance of a continuous and automated BMF requires about one-half of a man year at the technician level, tremendous cost savings for an industry may be realized over a relatively short time.

There are several other advantages to using a BMF. First, the system offers continuous monitoring protection. Second, the computer makes an objective decision regarding water or wastewater quality. Third, the BMF could detect a hazardous discharge prior to it entering a receiving system or it could detect when a drinking water supply has been contaminated. Ultimately, the use of a BMF would enhance the public's confidence in a treatment plant's operation.

There are several potential disadvantages to using the present BMF. First, only a single species is monitored. Whether this approach extends protection to other species remains to be seen. The literature does suggest, however, that the single species test with a sensitive life stage of fish may be a valuable component of an overall hazard assessment strategy. Second, it is difficult to determine the ecological significance of a fish changing its ventilatory rate. Although ventilatory rates change prior to acute mortality in the laboratory, the same may not be true in the field.

A third disadvantage is the initial capital outlay. Costs for the system's components, as described here, approach $65,000 (although the equipment may be phased in to reduce financial burdens). However, the potential for chemical monitoring trade-offs, coupled with a small operational budget, should make this approach cost-effective.

The fourth disadvantage to this automated and continuous monitoring approach is its experimental nature. To date, only Biological Monitoring, Inc.'s system is computer based and operated on-line. Several non-computer based systems are in operation at industrial sites and as research tools. However, most researchers have concluded that computers must be used to assess the large volumes of data. Some computer based systems were utilized for research purposes only.

Conceptually, the types of data provided by biological monitoring systems offer a more direct approach than physiochemical systems at predicting effluent toxicity. This study has illustrated the efficacy of this biological monitoring approach. The widespread acceptance of this approach, however, awaits regulatory approval at both the state and federal levels.

ACKNOWLEDGEMENTS

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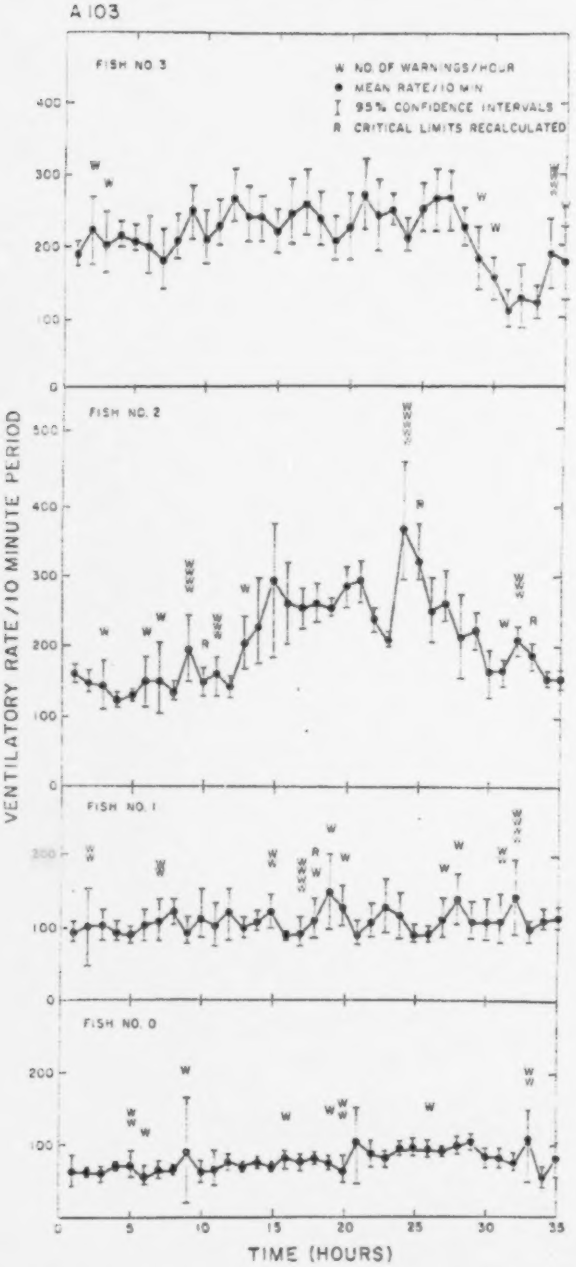

FIGURE 1. Ventilatory rate data for control (0 to 3) bluegill indicating warnings, means, and confidence intervals.

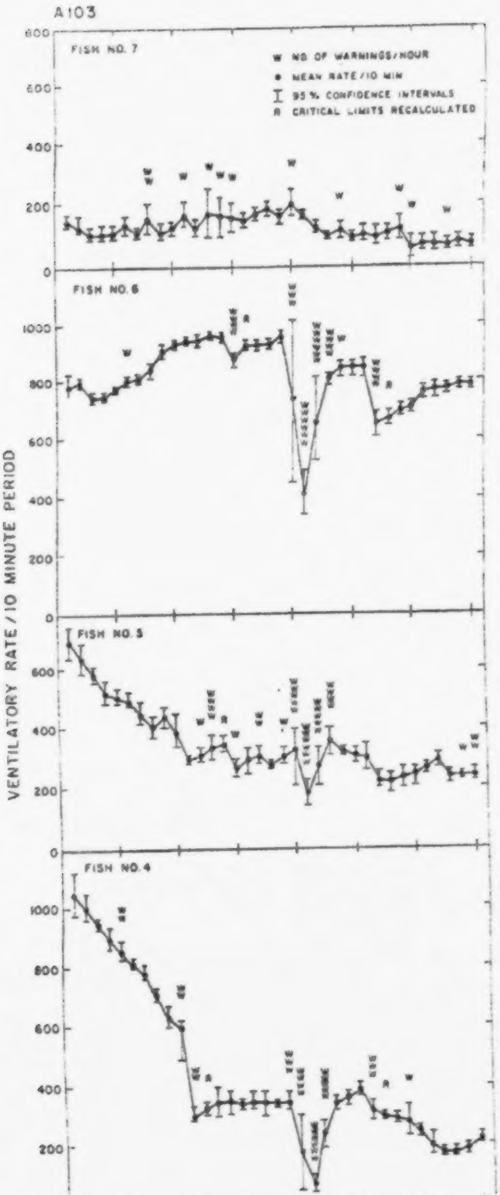

FIGURE 2. Ventilatory rate data for wastewater exposed fish. Wastewater was introduced at 19.3-hr and removed at 21-hr.

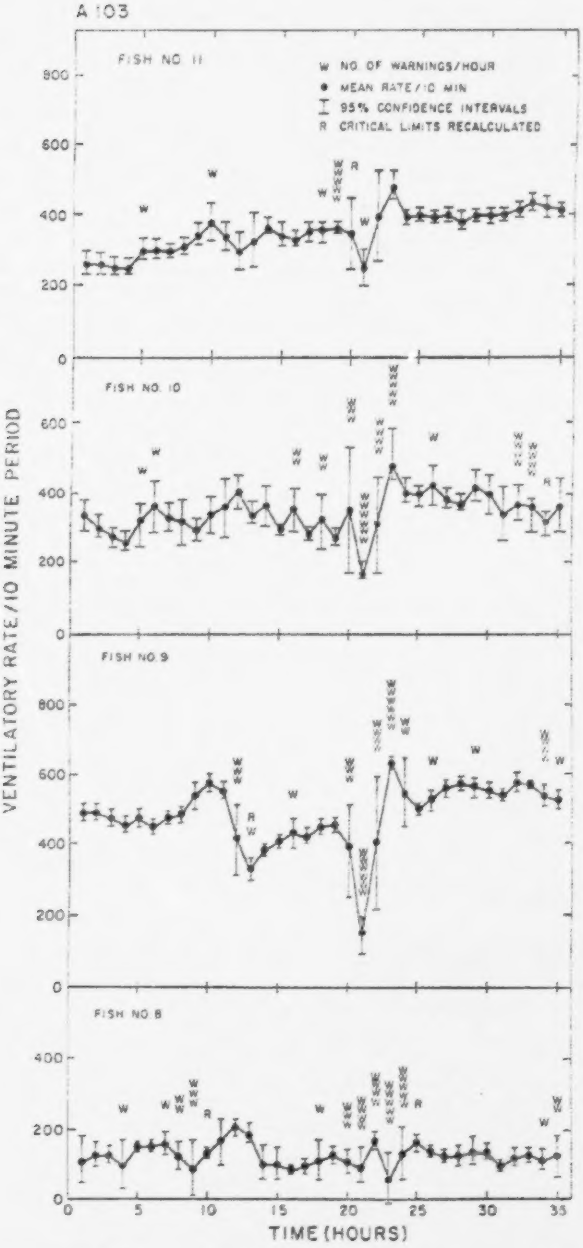

FIGURE 3. Ventilatory rate data for wastewater exposed fish. Wastewater was introduced at 19.3-hr and removed at 21-hr.

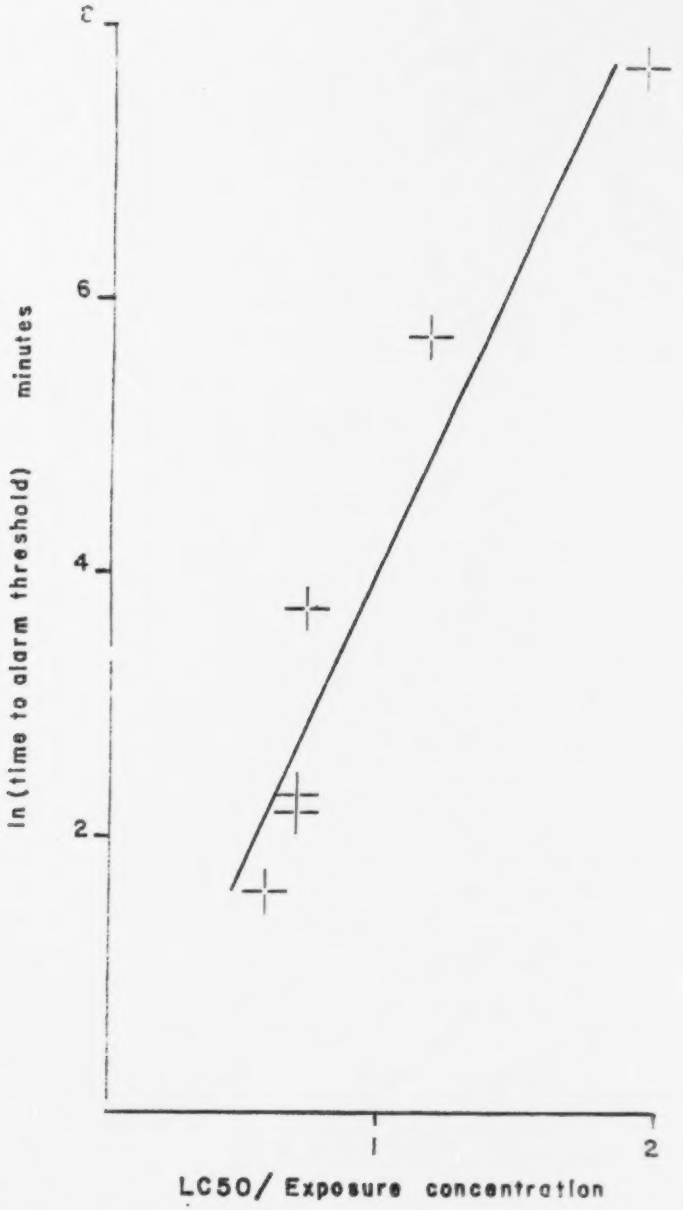

FIGURE 4. Relationship between 96-hr LC50 divided by ventilatory exposure concentration and the ln of time (minutes) to first high priority alarm.

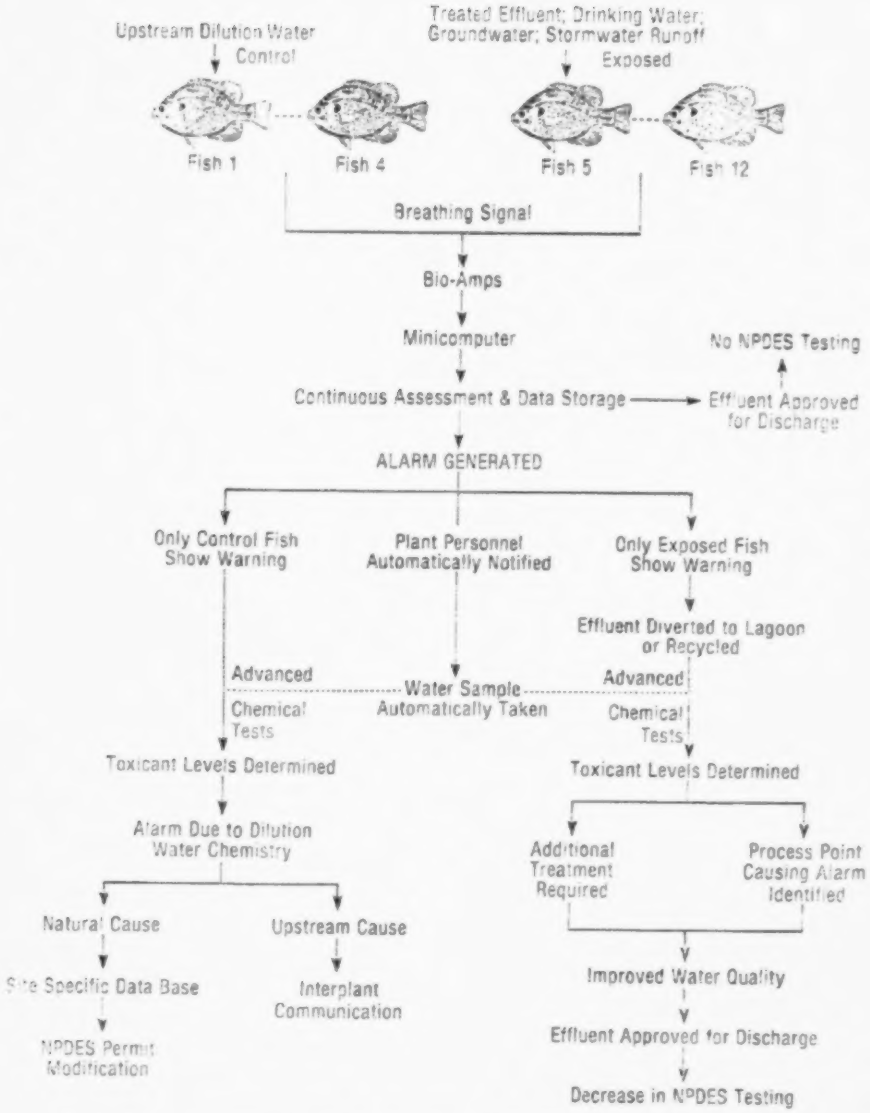
Upstream Dilution Water
Control
Treated Effluent; Drinking Water; Groundwater; Stormwater Runoff
Exposed
Fish 1
Fish 4
Fish 5
Fish 12
Breathing Signal
Bio-Amps
Minicomputer
Continuous Assessment & Data Storage
Effluent Approved for Discharge
No NPDES Testing
ALARM GENERATED
Only Control Fish Show Warning
Plant Personnel Automatically Notified
Only Exposed Fish Show Warning
Effluent Diverted to Lagoon or Recycled
Advanced
Water Sample Automatically Taken
Advanced
Chemical Tests
Chemical Tests
Toxicant Levels Determined
Toxicant Levels Determined
Alarm Due to Dilution Water Chemistry
Additional Treatment Required
Process Point Causing Alarm Identified
Natural Cause
Upstream Cause
Site Specific Data Base
Interplant Communication
NPDES Permit Modification
Improved Water Quality
Effluent Approved for Discharge
Decrease in NPDES Testing

FIGURE 5. Information flow within a Biological Monitoring Facility (BMF).

TABLE 1. A summary of acute bioassay and fish ventilatory data illustrating the correlations between LC50 and time to alarm.

EXPERIMENT	SAMPLE LOCATION	96-hr LC50 (%)[a]	VENTILATORY WARNINGS PRIOR TO SAMPLE		PERCENT[b] EXPOSURE	VENTILATORY WARNINGS AFTER SAMPLE INTRODUCTION		TIME TO ALARM AFTER SAMPLE INTRODUCTION (MINUTES)	
			HIGH	LOW		HIGH	LOW	HIGH	LOW
A	Influent to EB[c]	36	0	0	50	15	3	40	10
B	Influent to EB	58	1	0	30	1	1	2310	70
C	RBC Influent	35	0	0	50	3	8	10	250
D	RBC Influent	35	0	0	50	9	6	9	20
E	Final Effluent	29	0	1	50	1	428	5	15
F	Final Effluent	35	0	0	30	11	27	290	20

[a] As determined by Spearman-Karber analysis

[b] Percent dilution of sample with upstream dilution water

[c] Equalization Basin (EB)

BIOACCUMULATION OF MERCURY IN AQUATIC ECOSYSTEMS

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HAMMER, U. Theodore, P.M. HUANG and W. LIAW. 1982. Bioaccumulation of mercury in aquatic ecosystems. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The study was designed to determine the extent to which aquatic fauna and flora accumulated mercury and the relationship to mercury occurrence in sediments where organisms reside. This would form the basis for determining the biological role of mercury cycling in aquatic ecosystems. Benthic samples were taken by dredge throughout the upper portion of the Qu'Appelle River system. Littoral samples were also taken and seine hauls of small fish were made. Organisms and sediments were analyzed for mercury content subsequently. Every organism sample analyzed contained mercury. Concentrations over 1μg $Hg.g^{-1}$ dry weight were found in *Chironomus plumosus*, *Hyallela azteca*, *Corixidae* and *Gastropoda*. Somewhat lower values were recorded for other organisms including small fish, aquatic macrophytes and filamentous algae. No relationship to sediment mercury content was found. Biomagnification of mercury in the food chain was not apparent.

Key Words: Mercury, *Chrionomus plumosus*, *Hyallela azeteca*, *Corixidae*, *Gastropoda*, benthic invertebrates, fish.

HAMMER, U. Theodore, P.M. HUANG and W. LIAW. 1982. Bioaccumulation de mercure dans les écosystèmes aquatiques. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'étude vise à déterminer dans quelle mesure la faune et la flore aquatiques accumulent le mercure ainsi que le rapport entre cette accumulation et la présence de mercure dans les sédiments où résident les organismes en question. Les résultats obtenus permettent de déterminer le rôle biologique du mercure dans les écosystèmes aquatiques. On tire des échantillons benthiques de la partie supérieure du réseau fluvial Qu'Appelle au moyen d'une drague. On prélève aussi des échantillons le long du littoral et l'on pêche des quantités de petits poissons à la seine. On analyse les organismes et les sédiments ainsi recueillis afin d'en établir la teneur en mercure. Tous les échantillons analysés contiennent du mercure. On relève des concentrations de plus de 1 μg $Hg.g^{-1}$ de matière sèche dans les spécimens de *Chironomus plumosus*, d'*Hyallela azteca*, de corisidés et de gastéropodes. Les autres organismes, y compris les petits poissons, les macrophytes aquatiques et les algues filamenteuses, ont des concentrations légèrement inférieures. On ne peut démontrer aucun rapport entre la concentration de mercure dans les sédiments et la teneur en mercure des biotes. Il ne semble pas y avoir amplification de la concentration lorsqu'on remonte la chaine alimentaire.

INTRODUCTION

Mercury became recognized as an environmental problem in Japan in the 1950s and subsequently in Sweden in the 1960s. The problem became a North American one when Wobeser et al. (1970) found high mercury levels in fish of the Saskatchewan River system. Subsequently mercury of varying levels was found in food fish throughout Saskatchewan and North America. Maximum levels for human consumption were set at 0.5 ppm Hg. In some cases this resulted in the banning of fishing for commercial purposes. The Fish for Fun program was instituted in some Saskatchewan waters where mercury concentration in fish exceeded the limit. One of the systems affected was that of the Qu'Appelle River.

The Qu'Appelle basin includes 50,000 km^2 in southern Saskatchewan and one-third of the provincial population (Hammer, 1973). Lakes in the basin have multiple usage. Buffalo Pound Lake provides water for Moose Jaw and Regina. All the lakes have intensive recreational use including game fishing for walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*). The mercury content of these fish has often exceeded 0.5 ppm Hg since the early 1970s although other fish species have contained lower amounts. What is the source of the mercury?

Canada and Saskatchewan Departments of the Environment investigated stream sediments (Gummer and Fast, 1979) and found variations from 0.1 to 0.3 ppm total Hg (near background) to 38 ppm in stream sediments to 89 ppm in sediments along stream banks. The highest values occurred in the lower reaches of Thunder Creek and downstream in the Moose Jaw River. Few methyl mercury analyses were conducted but a range of 21 to 52 ppb was measured.

Gummer (1980) investigated Hg movement during spring runoff in 1979. Maximum suspended total Hg (0.13 µg Hg/L water) occurred at peak flow and maximum suspended sediments and particulate carbon levels. But the maximum in suspended sediment (0.58 ppm Hg) occurred when the clay and silt ratio was 1 which was prior to peak discharge. Otherwise the level was 0.15 ppm Hg. Loading of mercury ranged from 92 g day (April 16) at the start of runoff to 745 g Hg/day at peak discharge (April 24) to 42 g/day at runoff termination (May 22).

Munro and Gummer (1980) investigated biological uptake in Thunder Creek in a high mercury zone and 2.5 km upstream. There was a significant difference in mercury content between the two sites in the crayfish, *Orconectes virilis* (0.22 and 0.08 mg Hg/kg wet weight), the brook sticleback (*Culaea inconstans*) (0.15 and 0.09 mg Hg/kg), and pearl dace (*Semotilus margarita*) (0.07 and 0.03 mg Hg/kg). All of these species serve as prey for game fish, birds (e.g., great blue heron) and mammals (e.g., mink).

Rogers et al. (1981) showed that Qu'Appelle River system sediments sorbed and desorbed total mercury according to multiple first order kinetics. They found that these sediments have a high sorption capacity for Hg while the desorption process is relatively slow.

The objectives of our survey were to:

1. determine the distribution of mercury in sediments of the upper Qu'Appelle system.
2. determine the bioaccumulation of mercury in fauna and flora.
3. determine the relationship between sediment mercury and the amounts accumulated in the aquatic food chain.

METHODS

Sediment samples were collected from lakes and streams of the Qu'Appelle River system. During a preliminary survey in July 1978, sediments were collected from a broad spectrum of stream sites some of which are shown in Fig.1.

Subsequently sediments were collected in October 1980 from Buffalo Pound Lake (stations 1 and 2) near the east end of the lake and from the Moose Jaw River (stations 27 and 32). In May 1981 samples were collected from selected sites in part depicted in Fig.2. During September 1981, further samples were collected from Buffalo Pound, Pasqua and Katepwa lakes and at the Wascana-Qu'Appelle junction. Organisms were sieved from the sediments and identified. Organisms were also collected from the littoral even when the substrate there consisted of stones and coarse gravel. A seine was used to collect small fish.

Sediments from four sites, two in eastern Buffalo Pound Lake (BPL 1 and 2) and two in the Moose Jaw River (station 27 just above and station 32 just below Moose Jaw), were used in a laboratory experiment to determine the toxicity of sediments to *Chironomus plusmosus* larvae. Sediments were placed in pint jars and covered with distilled water. Chironomids from Buffalo Pound Lake containing 1.1 (BPL 1) and 0.58 (BPL 2) µg Hg/g dry weight were used in the experiments. Two control replicates were run with each of the native sediments (BPL 1 and 2) and four replicates each of sediments from Moose Jaw River stations 27 and 32 were established. Thirty to forty chironomids from either BPL 1 or BPl 2 were placed in each replicate. The experiments were run at 4°C (winter sediment temperature) for 120 days. Samples were examined at 40-day intervals to determine survivorship. Mercury analysis of the survivors was performed at the end of the experiments.

Sediments were stored at 4°C. Prior to chemical analysis sediments were thoroughly mixed. Analysis for total Hg was carried out with a UV mercury analyser (at the wavelength of 2.537 Å) utilizing the cold vapour technique for total Hg determination (Bishop, 1971; USEPA, 1974). Total organic carbon was also determined for the 1978 samples (Liaw and Huang, 1979).

RESULTS

Figure 1 illustrates the downstream variations of total mercury in sediments during July 1978. The highest values were in Wascana Creek below Regina but above the sewage disposal point source (1.381 µg Hg/g) dry weight of sediments), 0.501 µg/g in the Moose Jaw River below Moose Jaw and 0.314 µg/g in the Qu'Appelle River below its confluence with the Moose Jaw River. The lowest mercury levels were below Katepwa Lake (0.004), above Pasqua Lake (0.011), immediately below Buffalo Pound Lake (0.026), in Wascana Creek above Regina (0.027), and in the Moose Jaw River above Moose Jaw (0.034 µg Hg/g). The organic carbon content of these stream sediments ranged from 0.29 to 4.75%. There was a highly significant correlation between total mercury and organic carbon percent content of the sediments, r =0.664 (0.05, 25 d.f.).

Table 1 gives the variations in mercury content of sediments fauna and flora during 1980-81. Sediment mercury ranged from 0.089 (Buffalo Pound Lake off the provincial park) to 4.6 µg/g sediment dry weight at 19 m depth in the channel off Katepwa Point, Katepwa Lake. Concentrations in invertebrates ranged from <0.05 (Sphaerium) to 2.0 µg/g Hg/g dry weight (Corixidae). In fish muscle mercury concentrations varied from <0.05 (several species) to a maximum of 0.97 µg/g in *Culaea inconstans*. Macrophytes ranged between 0.03 (*Myriophyllum*) and 0.53 µg/g (*Scirpus*) while filamentous algae varied from 0.05 to 0.65 µg Hg/g (*Cladophora*). The correlation coefficient of the relationship between faunal and sediment mercury was 0.022 (0.05, 26 d.f.) while that for aquatic plants and sediment mercury was 0.723 (0.05, 3 d.f.). Neither relationship was significant. Only organisms directly associated with the sediments where mercury was measured were used in the correlations. Similarly correlations between *Chironomus plumosus* and Corixidae and sediment mercury proved nonsignificant. The variance for *C. plumosus* was 0.31 µg Hg/g (n = 6) while that for Corixidae was 0.37 µg Hg/g (n = 5).

Figure 2 shows some selected comparisons with respect to fauna and sediment mercury in the Qu'Appele system. The highest faunal mercury concentrations were in *Hyalella azteca* (1.40) and Corixidae (2.0) at Sandy Beach, Katepwa Lake, and in *Chironomus plumosus* (1.1 µg/g) in Buffalo Pound Lake in sediments with the lowest Hg concentration (0.089). Figure 3 shows scattergrams of the mercury concentrations in organisms compared with those of associated sediments.

Results for the laboratory experiment are given in Table 2 and Figs. 4 and 5. Figure 4 illustrates the differences in survival of chironmids from Buffalo Pound Lake (BPL 1) over 120 days in sediments from BPL 1 and Moose Jaw River sites 27 and 32. BPL 1 sediments served as a control. The use of a paired test showed that the numbers of survivors in sediments from the Moose Jaw River sites differed significantly from numbers in control replicates. The LD_{50} for site 32 averaged 92 days while LD_{50}'s for chironomids in the other two sediment types were not reached before the experiment was terminated. Figure 5 shows the trend for survival of chironomids from BPL 2 in sediments from BPL 2, site 27 and site 32. There was no significant difference in numbers of survivors in BPL 2 and site 27 sediments after the first 40 days. All other comparisons showed a significant difference between the controls (BPL 2) and experimental replicates for sites 27 and 32. LD_{50}'s were reached after 88 days in site 32 sediments and in 102 days in site 27 sediments but not in the control BPL 2 sediments during the 120 day experiment. There was a significant difference in chironomid survival between sites 27 and 32 with chironomids from BPL 1 but no significant difference with chironomids from BPL 2. There was no significant difference between sites BPL 1 and BPL 2 regarding chironomid survival.

Behaviour of the chironomids varied in the different sediments. In Buffalo Pound Lake sediments they tunnelled throughout the sediments and rarely appeared on the surface. The Moose Jaw River sediments were coarser (sand). In site 27 sediments there was some tunnelling but larvae tended to remain on the surface. In site 32 sediments little tunnelling was observed so larvae remained on the surface.

Lake and stream sediments were very fine grained (find silt and clay). The exceptions were sites 27 and 32 in the Moose Jaw River which contained some sand and the Sandy Bay, Katepwa Lake, 18 m deep site which had many mollusc shells interpersed with sediments. Oscarson et al. (1981) discuss the structure of these sediments in greater detail.

DISCUSSION

No overall downstream trend is evident with respect to total mercury concentration in Qu'Appelle River system sediments (Fig.1, Table 1). Headwater streams have low concentrations but these concentrations are higher than those above Pasqua and below Katepwa lakes. Very high concentrations of mercury are present below Moose Jaw and Regina implicating these cities as mercury sources. In part this has recently been verified as a large bed of concentrated mercury sediments has been located and excavated from the Moose Jaw River in Moose Jaw (D.T. Waite, pers. comm.). To date, no specific highly concentrated mercury beds have been found in the Regina area.

Jackson and Woychuk (1981) found 0.35 to 66 µg Hg/g in sediments of the Wabigoon River, Ontario, with an exponential decrease of total mercury downstream from the original source. In the Qu'Appelle River system there is also a decrease in mercury downstream in the lotic system below the cities. However, much higher concentrations occur in the downstream lakes (e.g., Pasqua, Katepwa [Table 1]). The lakes act as sinks for total mercury. Rogers et al. (1981) have shown that these lake sediments have a high capacity for the sorption of mercury. The presence of organic matter in Katepwa and Pasqua lake sediments increases their mercury absorptive capacity (Jackson, 1979). The significant correlation between percent organic carbon and mercury in sediments supports this, although adsorption would also take place to stream sediments, periodic flushing, particularly during spring runoffs, would erode mercury and sediments downstream. Jackson and Woychuk (1981) found that the solution of methyl mercury accelerated the loss of mercury from the sediments in streams.

The concentration of chloride in water may also have some influence regarding the sorption of mercury. Ramamoorthy and Rust (1978) found that a 10^{-4} M Nacl solution at neutral pH completely bound Hg^{2+} and sorbed most of the mercury from the sediments. Concentrations of chloride are much higher than this in the Qu'Appelle River lakes (e.g., 48 ppm in Buffalo Pound Lake, 161 ppm in Pasqua Lake) (Hammer, 1971). Concentrations of chloride in the streams would tend to be still higher below Moose Jaw and Regina (Warwick, 1966). Thus the binding qualities of the sediments must prevent the leaching of mercury from the sediments by the high chloride concentration in the aqueous medium.

Thomas (1974) found a range of 0.004 to 9.5 ppm total Hg in sediments of the Great Lakes with a mean of 0.347 ppm. He considered 0.083 ppm as mean background in Lake Superior. This is a greater range than we found in the Qu'Appelle River lakes.

Of particular interest is the considerable variation in mercury content in lake sediments between years and between seasons. For example, values in Buffalo Pound Lake were 0.089 and 0.12 in October 1980 and 0.39 and 0.57 µg Hg/g in May 1981 at stations 1 and 2, respectively, and 1.7 µg Hg/g at a nearby third site in September 1981. The sediments were located at about the same depth (ca. 2-3 m) on a relatively flat lake plain and appeared to be fairly similar in structure. If there are seasonal changes, we might expect the September sample to be more similar to the October concentrations rather than higher in mercury than any of the sites. On a flat lake plain, we might expect mercury to be distributed fairly evenly. In the channel off Katepwa Point in Katepwa Lake at a depth of 19 mm, mercury content of sediments was 1.0 in May and 4.6 µg/g in September 1981. Since the channel is relatively narrow (about 300 m) it is to be expected that currents may be of considerable importance in distributing heavy metals in sediments as occurs in streams (Gummer and Fast, 1979). In any case, it is almost certain that the source of mercury in these lake sediments is from outside the lakes themselves.

Temperature has been shown to affect the rate of mercury sorption and desorption (Rogers et al., 1981). However, bottom temperatures at the three periods of collection would vary little in Buffalo Pound Lake (8 to 12°C) but could be much colder in Katepwa Lake in deep water (6°C) where stratification occurs (Hammer, 1971).

Considerable variations in mercury content of organisms occurred in the system (Table 1, Figs. 2 and 3). Rooted aquatic vegetation accumulated <0.05 to 0.53 µg Hg/g dry weight. *Myriophyllum exalbescens*, *Potamogeton crispus*, and *Scirpus validus* accumulated maxima of 0.35 to 0.53 µg Hg/g. This contrasts sharply with Potter et al. (1975) who found that rooted plants accumulated relatively little mercury. We did not investigate whether the mercury was adsorbed on plant tissues from the water or translocated from the roots. Breteller et. al. (1981) found that *Spartina alternifolia* accumulated mercury at the highest concentrations in the roots. The accumulations they found growing on mercury treated sediments were similar to those we found in natural sediments. We did not determine mercury content of plant roots. Filamentous algae growing as Aufwuchs contained from <0.05 to 0.65 µg Hg/g. Presumably these algae would be more comparable to higher plants associated with the sediments than with phytoplankton. Potter et al. (1975) found similar values to the above in algae (28 ppm) and leaves (34 ppm) in Lake Powell, a desert reservoir. Philips (1977) summarized data showing accumulation in marine macroalgae of up to 20 ppm Hg. Blinn et al. (1977) found that photosynthesis of phytoplankton was inhibited when mercury > 0.06 ppm was added to contained subsystems of Lake Powell water and plankton.

Although the correlation between mercury in aquatic plants and associated sediments was not significant, the value r = 0.723 is fairly high. The low number of samples (n = 5) mitigated against a significant relationship. Brateler et al. (1981) found the plants tended to accumulate more mercury when the sediment content of mercury (artificially added) increased.

Most of the invertebrates analyzed for mercury content (Table 1) feed on plant material (living or dead). The clams (*Anodonta*, *Sphaerium*) are filter feeders in close association with sediments so that some of their food is probably associated with the sediment surface. Yet their flesh mercury content is only in the range of <0.05 to 0.25 µg/g. This corroborates the findings of Potter et al. (1975). Shells of *Anodonta* did not accumulate significantly more even though one might expect tissues to clear mercury more readily than would be likely with calcium carbonate shells. The accumulation rate for the shell is probably lower. This is likely true for gastropods as well. Karbe et al. (1975) found the largest mercury accumulation in clams and mussels in the Elbe River when oxygen was highest. In the Qu'Appelle system oxygen concentrations may be low only in deep waters of Katepwa Lake in the summer (Hammer, 1971).

Sediment feeders such as chironomids and oligochaetes accumulated considerable amounts of mercury (i.e., 0.34 to 1.1 µg/g). Their diet consists of organic material in the sediment and organic matter has been reported to accumulate relatively large amounts of mercury (Jackson 1979; Potter et al., 1975). However, organisms which do not feed on sediment *per se* such as amphipods and croxids have even higher mercury accumulations at times (Table 1, Figs. 2 and 3). Maximum values were between 1.4 and 2.0 µg H/g. These organisms feed on plant material on the surface of the sediments. In contrast to Armstrong and Hamilton (1973), the highest concentrations (2.0 µg Hg/g) occurred in littoral dwelling corixids remote from any fine sediments (i.e., on a sand-gravel-rock beach in Katepwa Lake). The amphipod *Hyalella* also had the highest concentrations (1.40 µg Hg/g) at this site. Profundal sediments nearby (300 m) at 18 m depth only contained 0.27 µg Hg/g.

Secondary consumers (small fish) for the most part had relatively low concentrations of mercury (i.e., <0.05 to 0.25). The only exception was for *Culaea inconstans* (0.97 µg Hg/g collected from Wascana Creek where the sediments contained 2.0 µg Hg/g). Crayfish (*Orconectes*) generally had higher or as much as the maximum of the fish they associated with. The biggest mercury concentration (Table 1) was in a large crayfish collected at Katepwa Point, May 1981. In September at the same location mercury concentrations in crayfish were the same as in small pike and perch and the amphipod *Hyalella* (i.e., 0.26 µg/g). The same levels were recorded for *Orconectes* and a 15 cm burbot (*Lota lota*) in Pasqua Lake. In contrast, in Buffalo Pound Lake at that time *Orconectes* contained 0.49 µg Hg/g, a pondweed *Potamogeton crispus* 0.46 µg/g while perch and two minnows (*Pimephales*, *N. atherinoides*) only had 0.05 µg Hg/g. It would therefore appear that crayfish contain at least as much mercury as do fish. Armstrong and Hamilton (1973) suggested this comparison between crayfish and pike mercury accumulation. Crayfish and fish are quite mobile and may thus be exposed to a broad spectrum of mercury concentrations if such exist in the ecosystem in which they live. Since crayfish perforce live on the sediments they may acquire more by mere proximity. Plants and most of the other invertebrates tend to have rather limited ranges from which they can accumulate mercury. Thus it might be expected that they might have concentrations of mercury much more closely related to their immediate environment than that of fish and crayfish to the environment in which they are caught.

Figure 3 illustrates the relationship between mercury concentrations of organisms with that of the sediments with which they were associated. No trend is obvious and this is borne out by non-significant correlation coefficients of 0.022 between fauna and 0.723 with flora and sediment mercury, respectively. Philips (1977) cites the inability of investigators to correlate metal concentrations in water or biota with those in sediments. With respect to health risks, the bioavailability of metals, in this case mercury, is the primary concern. It is generally accepted that most of the mercury present in organisms is in the form of methyl mercury and that it is mostly accumulated in this form (Bishop and Neary, 1974; Huckabee et al., 1975; de Freitas et al., 1974). Inorganic sediment mercury may be ingested directly if it is adsorbed to food items. It may, however, be converted by bacteria to methyl mercury (Jensen and Jernelov, 1969) which may then become available via the water to organisms. Philips and Buhler (1978) found that rainbow trout accumulated methyl mercury from both food and water. Johnels et al. (1967) found the pike accumulated mercury according to length, weight and age. Variations within other species according to a variety of parameters probably occur. Philips (1977) emphasizes the concentration of a metal in the sediment provide no data on the amount of the metal available to biota. Rogers et al. (1981) state that a number of factors influence the rate of mercury desorption including temperature, the specific minerals making up the sediments and the charges on the particles. Ramamoorthy and Rust (1978) stress the importance of the presence of strong leaching agents in order to mobilize heavy metals to any extent. Brateler et al. (1981) found that sediments with a high organic content allowed almost no bioaccumulation for resident biota. Thus a great number of variables associated with both the sediments and the organisms play roles in the final determination of mercury concentration in a specific organism.

There is no evidence of biomagnification of mercury in the food chain in the Qu'Appelle River system nor is there an increase in mercury concentration at higher trophic levels. The variations in each of the trophic levels tends to be fairly similar. In fact primary consumers have the highest mercury levels. This suggests that direct accumulation from the environment rather than through the food chain is probably the most important pathway for mercury bioaccumulation. Huckabee et al. (1975) showed that *Daphnia pulex* accumulated only 15%

of its mercury load from its food while Philips and Buhler (1978) found that rainbow trout accumulated methyl mercury from both food and water.

Potter et al. (1975) claimed that bioamplification of mercury in the food chain occurred in Lake Powell. There is little evidence for this other than that consumers contained higher concentrations of mercury than producers. They do not include any information on invertebrates other than crayfish which are not primary consumers. Their finding that increaased mercury concentrations were associated with greater body weight was not borne out between different species in our study. Experimental work must be carried out in order to assess mercury bioaccumulation in the food chain as natural systems are too complex to draw any precise conclusions. However, bioaccumulation involves both the food chain and the environment.

Experimental results showed that Chironomus plumosus continued to increase their mercury concentration with time in each of the microosms (Table 2). The only source of mercury was from the sediments. This could be obtained directly through ingestion of organic material where mercury tends to be concentrated (Reimers et al., 1974) or through gills and other body surfaces. In each case the concentration by the chironomids is higher than the original sediment concentration. The degree of concentration was less from sediments with higher original mercury content (i.e., Moose Jaw River) but this may be attributed to a lower degree of tunnelling and, perhaps, feeding.

With respect to survival, the original habitat sediments (Buffalo Pound Lake) ensured the highest survival rate (Figs. 4 and 5). These sediments also had the lowest initial mercury concentration. In spite of this,mercury concentration was highest in chironomids which survived 120 days in the sediments from Buffalo Pound Lake (8.8 μg/g) while mercury concentrations in the other cultures were much lower (Table 2). This may indicate that mercury per se was not responsible for the chironomic mortality but rather a combination of other factors inherent in the sediments. It may also indicate that other substances in the sediments may be antagonistic to Hg toxicity. Although selenium has been suggested it was as high or higher in the other sediments.

This study has done little to clarify the role of the food chain in the bioaccumulation of mercury at the various trophic levels. A follow-up study is being conducted to ascertain this role particularly regarding the final accumulation of mercury by fish. It must also be established as to which mercury fractions are being accumulated at each step of the food chain although it appears likely that methyl mercury is the major component.

CONCLUSIONS

1. No overall downstream trends in sediment total mercury are apparent.
2. There is considerable variability from season to season and year to year in sediment mercury at similar sites.
3. All species examined accumulated mercury but concentration rates differed within and between species.
4. There was no relationship between sediment and organismal total mercury.
5. Biomagnification of mercury in the food chain was not apparent.

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Table 1. Range of concentrations of Hg, (µg/g dry weight) in faunal and floral species and sediments of the upper Qu'Appelle River system.

	Hg
Invertebrates	
Gammarus lacustris	<0.10→0.20±0.04
Hyalella azteca	<0.25→1.40
Orconectes - flesh	<0.25→0.93±0.05
- shell	<0.10→0.48±0.03
Sphaerium	<0.05
Anodonta - flesh	0.17→0.25±0
- shell	0.14→0.40
Gastropoda - flesh	1.2
- shell	0.8→1.1
Hirudinea	0.30±0.01
Oligochaeta	0.73±0.02
Corixidae	0.32→2.0±0.2
Chironomus anthracinus	0.35±0.05→0.63
Chironomus plumosus	0.34±0.05→1.1
Notonectidae	0.32
Trichoptera	0.39
Fish	
Culaea inconstans	0.97±0.02
Esox lucius - flesh	0.26±0.06
- bones	<0.05
Etheostoma exile	<0.25
Lota lota - flesh	<0.25
- bones	<1
Notropis atherinoides	
- flesh	0.05±0→0.25±0
- bones	0.11±0.01
Notropis hudsonius	
-flesh	<0.10
-bones	<0.25
Perca flavescens	
- flesh	<0.05→0.26±0.02
- bones	0.2±0.02
Pimephales promelas	<0.05→0.14±0
Pungitius pungitius	0.30±0

	Hg
Flora	
Cladophora crispata	<0.05→0.65±0
Enteromorpha intestinalis	0.44±0.05
Ulothrix sp.	0.48±0.03
Myriophyllum exalbescens	0.03±0.01→0.35±0.05
Potamogeton crispus	0.46±0
Potamogeton pectinatus	<0.05
P. richardsonii	0.10±0.03
Scirpus validus	0.32±0.02→0.53±0.04
Sediments	-

Buffalo Pound Lake	Oct. 80	May 81	Sept. 81
Station 1	0.089	0.39	-
Station 2	0.12	0.57	-
Station 3	-	-	1.7±0
Moose Jaw River	1.1-1.5	0.51	-
Qu'Appelle River	-	0.43	-
Wascana Creek			
Station 1	-	2.0±0.1	-
Station 2	-	5.4±0.1	-
Pasqua Lake			
Station 1	-	2.6±0.1	-
Station 2	-	2.2±0.1	-
Pasqua-Echo Channel	-	1.2±0	-
Katepwa Lake			
Sandy Beach	-	0.27	-
Katepwa Point	-	1.0±0.2	4.6±0.2

Table 2. Stations as source of experimental sediments and mercury concentrations (µg Hg/g dry weight) in sediments and *Chironomus plumosus* when the experiment was terminated.

Station	Sediments	*Chironomus plumosus*	Terminal
Buffalo Pound Lake 1	0.089		8.8
Buffalo Pound Lake 2	0.12		1.6
Moose Jaw River 27	1.1	BPL 1	1.5
		BPL 2	2.4
Moose Jaw River 32	1.5	BPL 1	1.6
		BPL 2	2.0

List of Figures

Figure 1. Map of the study area. The numbers represent micrograms total mercury per kilogram sediments.

Figure 2. Concentrations of total mercury (µg/g dry weight) in the Qu'Appelle River system.

Figure 3. The relationships of organismal and sediment total mercury in the Qu'Appelle River system. Fig. A - fauna:

A_s^f - *Anodonta* (flesh, shell), Co-Corixidae, Ca-*Chironomus anthracinus*

Cp - *C. plumosus*, Gl-*Gammarus*, Ha-*Hyalella*

O_s^f - *Orconectes* (flesh, shell), S - *Sphaerium*

Fig. B. C-*Cladophora*, E - *Enteromorpha*, M - *Myriophyllum*

Pc-*Potamogeton crispus*, Pp - *P. pectinatus*, Pr - *P. richardsonii*

S - *Scirpus*

Figure 4. The survival time of chironomids from Buffalo Pound Lake Station 1 in sediments from that station and in sediments from Stations 27 and 32 in the Moose Jaw River. I represents the range of organism numbers still alive on the specific date.

Figure 5. The survival time of chironomids from Buffalo Pound Lake Station 2 in sediments from that station and in sediments from Stations 27 and 32 in the Moose Jaw River. I represents the range of organism numbers still alive on the specific date.

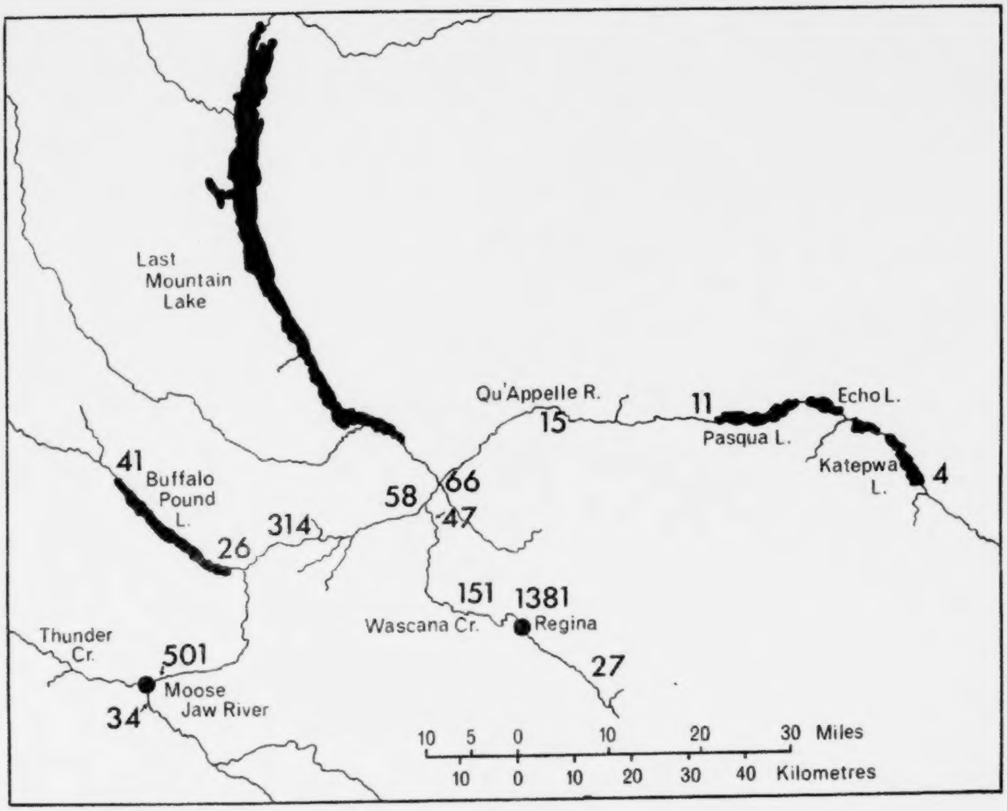
Last Mountain Lake
Qu'Appelle R.
15
11
Echo L.
Pasqua L.
Katepwa L.
4
41
Buffalo Pound L.
58
66
47
314
26
151
1381
Wascana Cr.
Regina
27
Thunder Cr.
501
Moose Jaw River
34
10 5 0 10 20 30 Miles
10 0 10 20 30 40 Kilometres

Figure 1

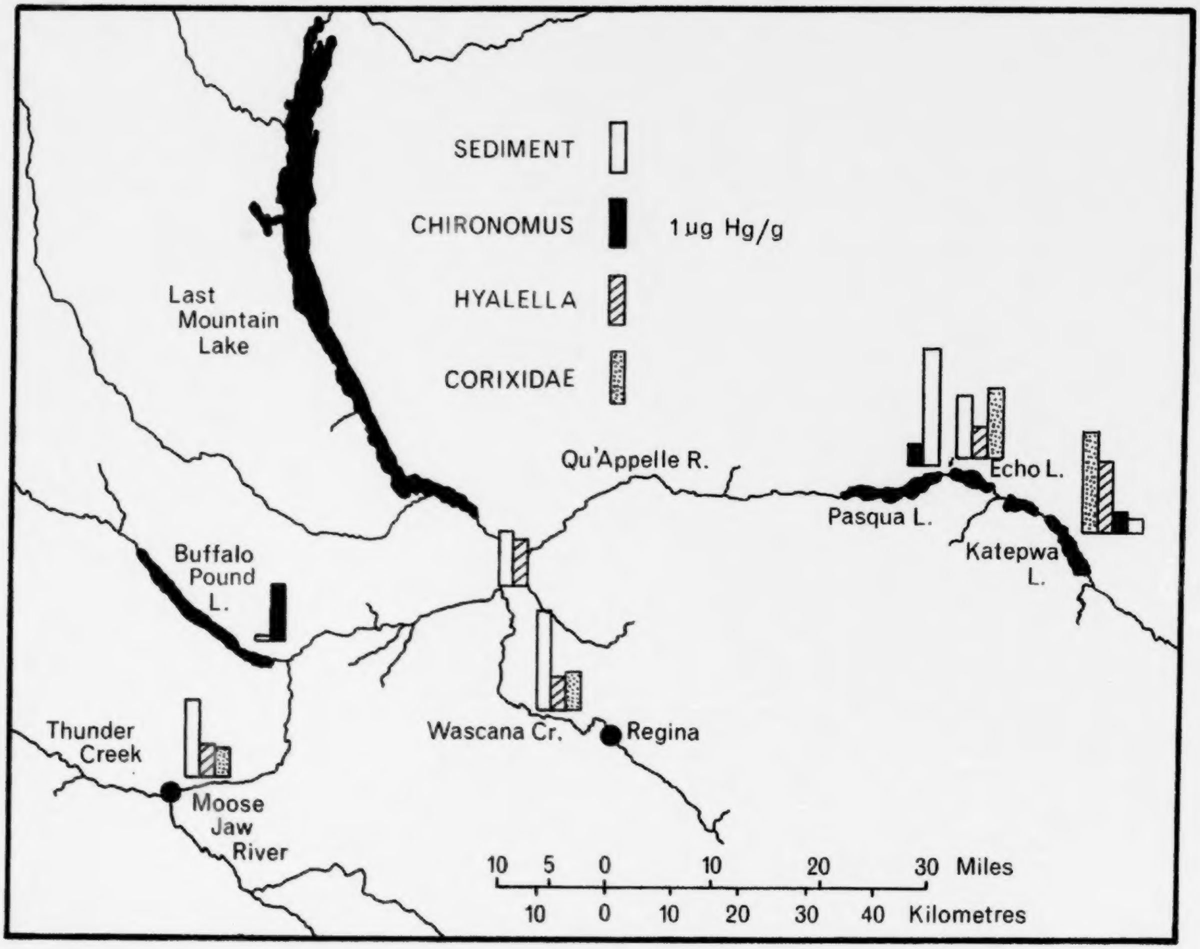
SEDIMENT
CHIRONOMUS
HYALELLA
CORIXIDAE
1 µg Hg/g
Last Mountain Lake
Buffalo Pound L.
Qu'Appelle R.
Pasqua L.
Echo L.
Katepwa L.
Thunder Creek
Moose Jaw River
Wascana Cr.
Regina
10 5 0 10 20 30 Miles
10 0 10 20 30 40 Kilometres

Figure 2

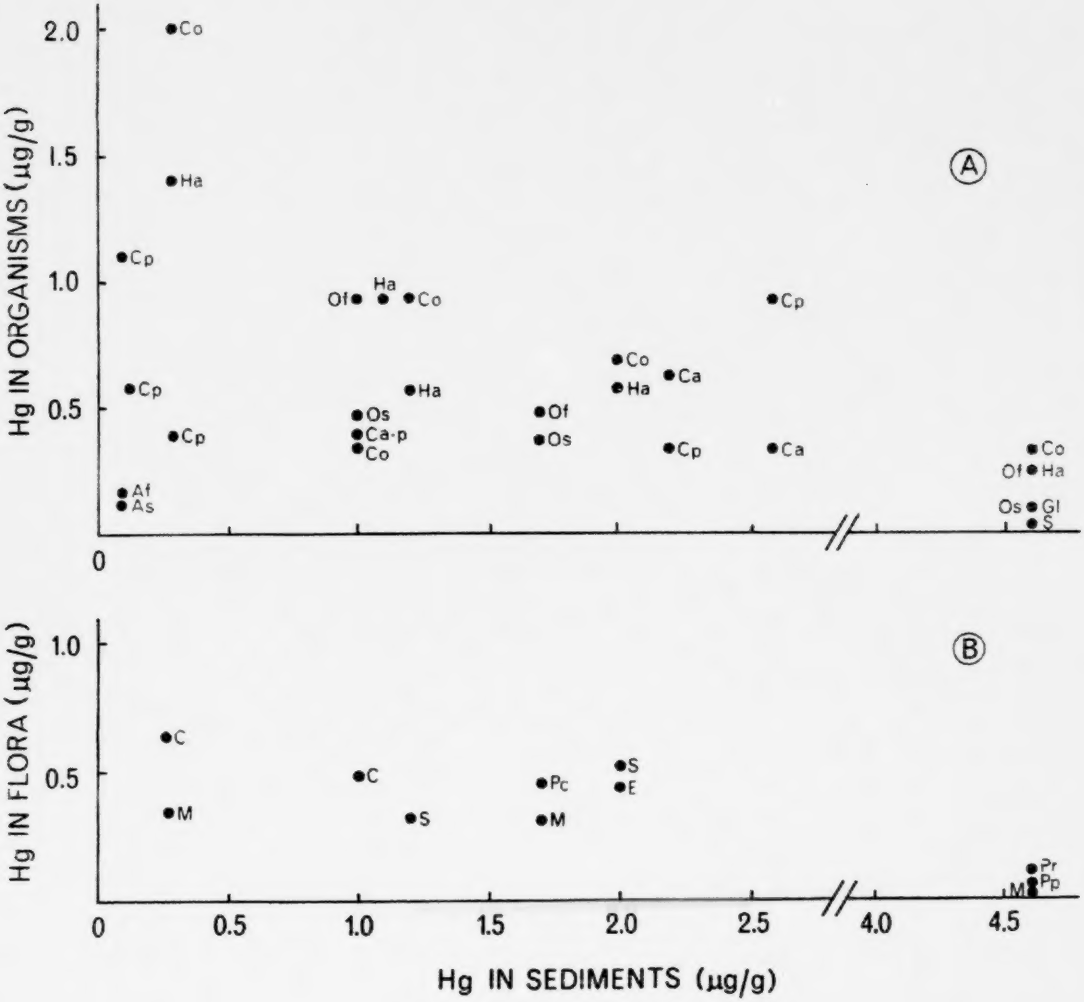
Hg IN ORGANISMS (µg/g)
2.0
1.5
1.0
0.5
0
Co
Ha
Cp
Cp
Cp
Af
As
Of
Ha
Co
Ha
Os
Ca-p
Co
Of
Os
Co
Ha
Ca
Cp
Cp
Ca
Co
Of
Ha
Os
Gl
S
A
Hg IN FLORA (µg/g)
1.0
0.5
C
M
C
S
Pc
M
S
E
Pr
Pp
M
B
0
0.5
1.0
1.5
2.0
2.5
4.0
4.5
Hg IN SEDIMENTS (µg/g)

Figure 3

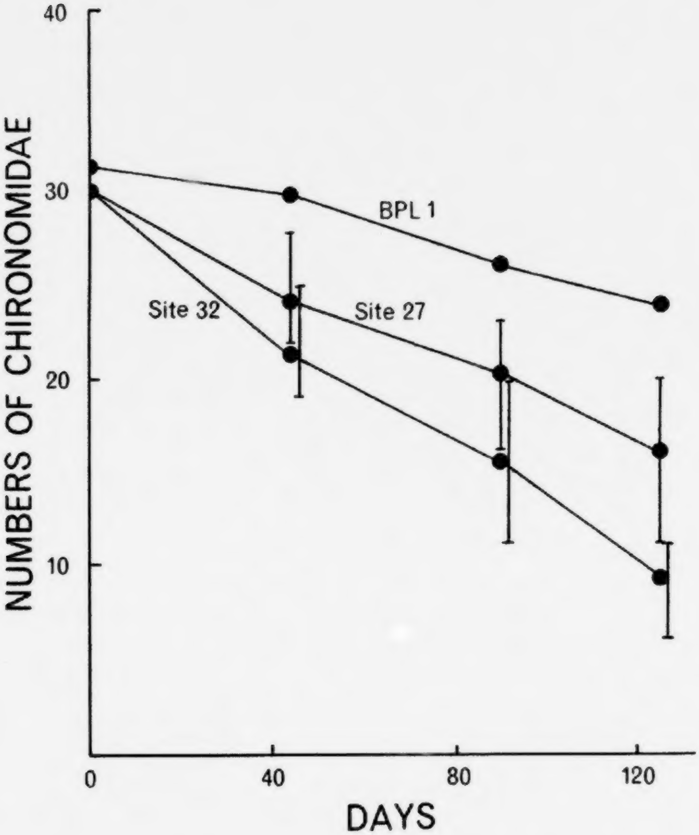
NUMBERS OF CHIRONOMIDAE
40
30
20
10
0
40
80
120
DAYS
BPL 1
Site 32
Site 27

Figure 4

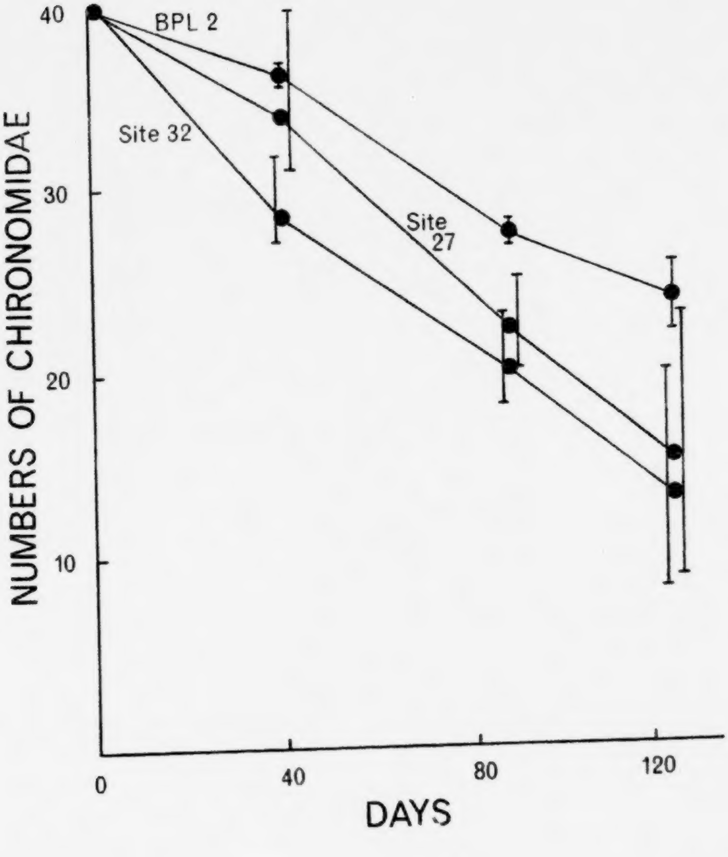
40
30
20
10
NUMBERS OF CHIRONOMIDAE
BPL 2
Site 32
Site 27
0
40
80
120
DAYS

Figure 5

PHYSICAL AND BIOLOGICAL DYNAMICS OF CRUDE OIL AND ITS COMPONENTS

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HIGGINS, R.J., W.L. LOCKHART, B.N. BILLECK, R. DANELL and D.A.J. MURRAY. 1982. Physical and biological dynamics of crude oil and its components. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The water soluble fraction (WSF) of Dome Nektoralik crude oil was studied. Volatilization losses of the WSF components mixed with varying concentrations of Corexit 9527 (Esso) were analyzed by gas chromatography. Uptake and clearance of the WSF was determined in rainbow trout (Salmo gairdneri) and toxicity assayed in cisco (Coreganus artedii) and duckweed (Lemna minor). The WSF components volatilized quickly from water and Corexit had no effect on volatilization. Rainbow trout accumulated the lighter aromatic components of the WSF. The 96 hr. LC-50 for cisco was 1.5 to 2 ppm. Duckweed growth was unaffected by the oil unless an oil slick was present.

Key Words: Crude oil, water soluble fraction, Corexit 9527, uptake, clearance, rainbow trout, cisco, duckweed, growth, Coregonus artedii, Salmo gairdneri, Lemna minor.

HIGGINS, R.J., W.L. LOCKHART, B.N. BILLECK, R. DANELL and D.A.J. MURRAY. 1982. La dynamique physique et biologique du pétrole brut et de ses composantes.

On a étudié la fraction hydrosoluble (F H S) du pétrole brut du Dôme Nektoralik. Les pertes par volatilisation des composantes de la F H S mélangées à diverses concentrations de Corexit 9527 (Esso) ont été analysées par chromatographie en phase gazeuse. On a déterminé l'absorption et l'élimination de la F H S chez la truite arc-en-ciel (Salmo gairdneri) et la toxicité chez le cisco (Coreganus artedii) et la lentille d'eau (Lemna minor). Les composantes de la F H S se volatilisaient rapidement de l'eau et le Corexit n'avait pas d'effet sur ce processus. Les composantes aromatiques les plus légères de la F H S se concentraient chez la truite arc-en-ciel. La CL_{50} de 96 h chez le cisco se situait entre 1,5 et 2 ppm. Le pétrole brut n'avait pas d'effets sur la croissance de la lentille d'eau à moins qu'il se forme une nappe de pétrole à la surface de l'eau.

INTRODUCTION

The exposure of aquatic ecosystems to crude oils, refined petroleum products and refinery effluents has led to a wealth of data pertaining to the toxicology of oil components to various organisms. Less work has been performed utilizing the complete water soluble fraction(WSF) to which pelagic and benthic organisms are exposed in the environment. It is the objective of this paper to investigate some effects of the water soluble fraction of a Beaufort Sea Oil on both animals and a plant in laboratory tests and to study the chemical and biological effects of the interaction of an oil dispersant with the WSF.

METHODS AND MATERIALS

PREPARATION OF THE WSF

The Beaufort Sea crude oil was obtained in a tinted four litre glass container lacking any headspace. This sample was kept at room temperature prior to use. The water soluble fraction(WSF) of Beaufort Sea oil was prepared by vigorously shaking the crude oil with water dechlorinated by ultra-violet light in a 1 litre separatory funnel for 10 minutes and then allowing the mixture to stand for at least 48 hours before drawing off the aqueous phase. Varying amounts of oil(1-50ml) were mixed with one litre of water to determine the minimum amount of oil required to saturate the aqueous phase.

ANALYSIS OF THE WSF

Gas chromatographic determination of the hydrocarbon composition of the WSF was performed using a microextraction technique(Murray and Lockhart,1981) on either a Hewlett-Packard gas chromatograph(GC) using an SP 2100 fused silica WCOT column and a Flame-ionization detector or a Perkin-Elmer 900 GC with similar column and detector. Gas chromatographic headspace analysis was also performed by equilibrating equal volumes of extract and nitrogen for two minutes in a gas tight syringe. The headspace was then injected onto a 5% Dexsil column.

DISPERSANT AND THE WSF

The effect of the oil dispersant Corexit 9527(Esso) on the WSF was determined by adding varying amounts of the dispersant during preparation of the WSF. Analysis of the WSF and dispersant mixture by the microextraction and headspace technique were performed over three days.

UPTAKE BY RAINBOW TROUT

Rainbow trout(*Salmo gairdneri* Richardson) were exposed to a fifty percent saturated WSF in 2 litre glass tanks open to the air at 10 degrees Celsius. Trout were sacrificed at intervals over two days and tissue extracts were prepared for GC analysis as described by Murray and Lockhart 1981.

FISH TOXICITY

A 96-hour lethal toxicity test was performed on 0.27 gram cisco(*Coregonus artedii* Lesuer) fry. A multiple channel peristaltic pump was used to generate a flow-through bioassay in 2 - litre covered tanks. All-tubing tanks and covers were glass The five WSF concentrations tested were sampled twice daily by headspace analysis for total hydrocarbons. Both the Litchfield-Wilcoxan procedure and a Probit analysis were used to calculate the 96-hour LC-50.

PHYTOTOXICITY

The WSF toxicity to duckweed(*Lemna minor*) was tested by mixing varying dilutions of the WSF and Corexit 9527 with the Stewart's growth medium (Lockhart and Blouw, 1979). Duckweed frond counts were made at varying intervals over a period of 28 days. In another experiment the plants were exposed to a surface slick of the crude oil as well as the WSF.

RESULTS AND DISCUSSION

Oil researchers have long been aware of the necessity of studying the water soluble fraction of crude oils in order to assess their toxicity to aquatic organisms. Unfortunatly no standard method of preparing the WSF has developed. Variations in preparation range from an oil-water ratio of 1.6 with a mixing time of twelve hours and a separation time of two hours(Boylan and Tripp,1971)to a 1:1000 oil:water ratio with a ten - minute mixing time and the use of centrifugation for seperation(Kappeler and Wuhrmann 1978). The effectiveness of the ten-minute mixing time used in this study is evidenced by total hydrocarbon analysis(Fig.1)which shows a saturated aqeous phase. Unpublished work at our lab has indicated that after allowing 72 hours for seperation all particulate hydrocarbons have seperated out of the aqueous phase. The minimum seperation time used in our study was 48 hours. Thus small quantities of particulate hydrocarbons may have been present in some assays but not at levels which would have a significant effect.

As the Beaufort Sea oil was limited in volume it was necessary to determine the minimum amount required to generate a saturated water soluble fraction. Headspace analysis of the WSF prepared after using between 1 to 50 ml of oil in one litre of water indicated that volumes greater than 10 ml of oil failed to contribute significantly to the total hydrocarbons in the water(Fig.1). All water soluble fractions used in this report were therfore prepared using 10 ml of crude oil in one litre of water.

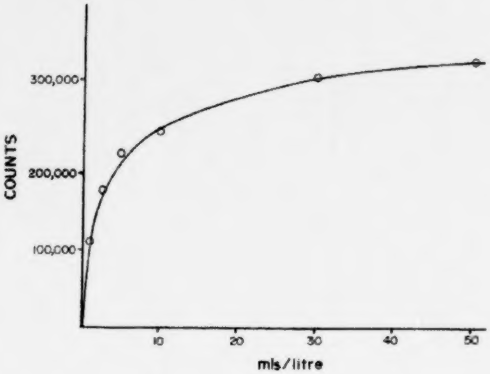

Fig.1 Integrator counts of oil components in the water phase after mixing varying quantities of oil with water and allowing the layers to separate.

As it was impossible to assess any alterations of the crude oil that may have occurred during shipping, a gas chromatograph was prepared prior to use of the oil(Fig 2a). In this way we quantified the nature of the oil received.

Gas chromatographic analysis of the Beaufort crude oil(Fig.2a) revealed the hydrocarbon profile of a mixed-base oil typical of Alaskian crudes(Payne 1981). The WSF prepared from this crude(Fig.2b) is rich in the more soluble low molecular weight alkanes and aromatics but almost totally lacking the heavier insoluble hydrocarbons beyond the dimethylnapthalenes. This correlates well with GC analysis of other WSF's prepared from similar crudes(Zurcher and Thuer.1978;Payne 1981).

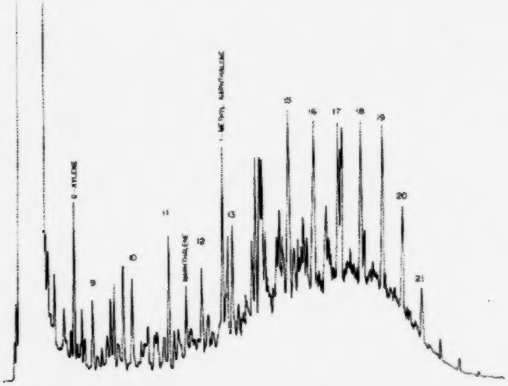

Fig. 2(a) Gas chromatograph of Beaufort Sea crude oil (1 200 dilution)

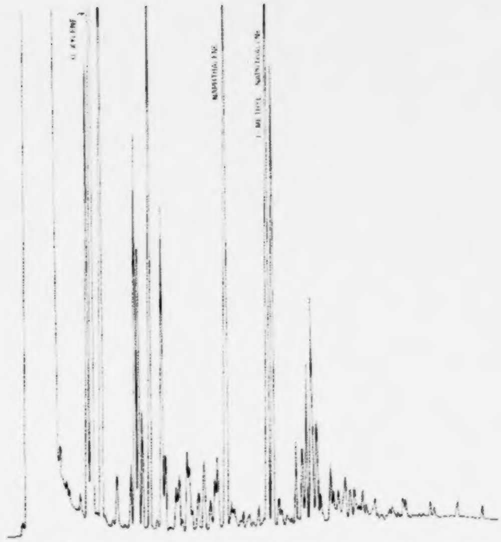

Fig 2(b) Gas chromatograph of the water soluble fraction of Beaufort Sea crude oil(prepared by microextraction).

The addition of Corexit 9527 at concentrations ranging from 0.1 to 10 ppm had little effect on losses of either volatile or non-volatile hydrocarbons(Table 1). This contrasts with the findings of Shaw and Reidy(1979) who reported a three-to seven-fold increase in emulsified hydrocarbons after the addition of Corexit 9527. We believe that our findings are the result of both the low concentrations of dispersant used and the decreased effectiveness of Corexit 9527 in freshwater as reported by Esso Chemicals(in Lonning and Hagstrom,1976). It should be noted however that Scott et al.(1979) were successful in using Corexit 9527 in freshwater to increase the amount of oil in water when using a 1:6 ratio of dispersant to oil(~170 parts per thousand). Unfortunatly Shaw and Reidy(1979) neglected to report the amount of dispersant used in their salt water tests, making comparisons impossible.

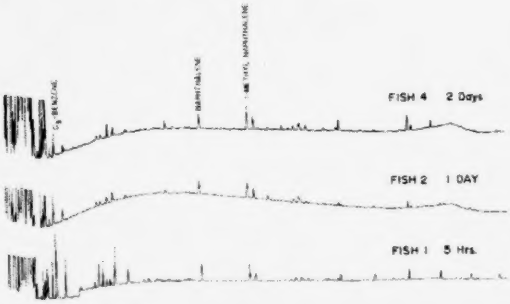

Fig. 3 Hydrocarbons found in fish exposed to water soluble fraction of Beaufort Sea crude oil.

Analysis of tissue extracts from rainbow trout exposed to the WSF for periods ranging from five hours to two days indicated an uptake of the light aromatics ranging from the xylenes, toluenes, and ethlybenzenes to the dimethlynapthalenes(Fig.3). This is similar to the experience of Kuhnhold et al. 1978. Greater concentrations of the alklyated smaller aromatics were recovered after five hours than after two days. This may be due to selective depuration of these toxic hydrocarbons with time.

The LC-50 for cisco fry exposed to the WSF ranging in concentration from 1.5 to 6.4 ppm total hydrocarbons(Fig.4) was calculated by both the Litchfield-Wilcoxan procedure and Probit analysis. The 96-hour LC-50 using the Litchfield modification of the Probit analysis was 1.54 ppm(Fig.5). The 96-hour LC-50 based on a standard Probit analysis was 1.99 ppm with fiducial limits of 1.48-2.55 ppm. These results compare favourably with literature regarding other oils(Craddock, 1977;Rice et al., 1979; Moles et al., 1979).

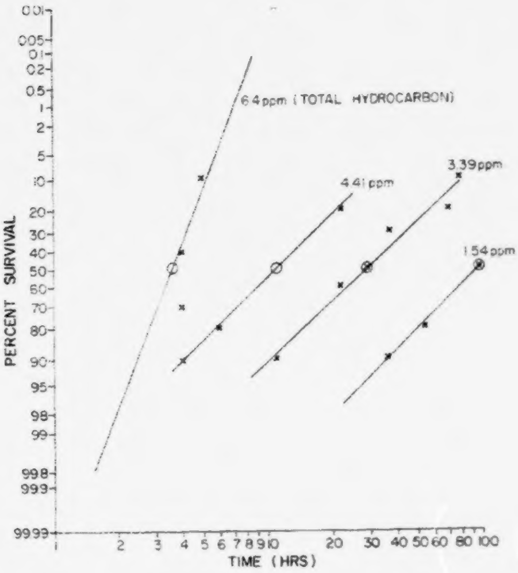

Fig. 4 Mean survival times of cisco fry exposed to measured concentrations of the water soluble fraction of Beaufort Sea crude oil.

Headspace analysis for total hydrocarbons revealed considerable variation in the concentration of the WSF during the cisco fry flow-through bioassay (Fig.6). This illustrates the difficulties encountered when running a flow-through system with such a complex mixture of hydrocarbons. Great care must be taken to minimize contact of the solution with adsorbtive surfaces and any headspace which will allow loss of volatiles. Such losses would alter the hydocarbon profile and thus affect our knowledge of the hydrocarbons present in exposures.

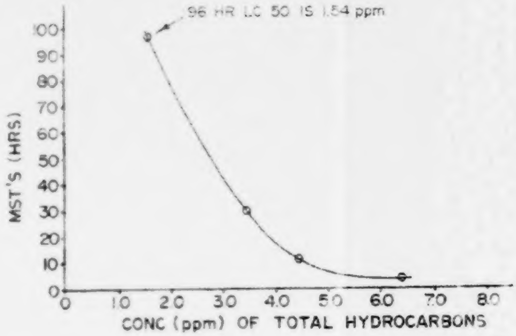

Fig. 5 Derivation of 96-hour LC-50 for cisco fry exposed to water soluble fraction of Beaufort Sea crude oil(MST=Mean Survival Time)

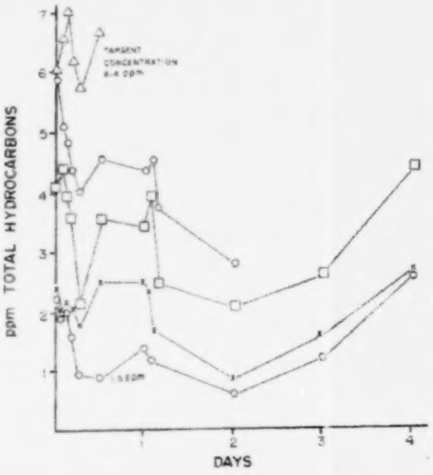

Fig. 6 Measured quantities of hydrocarbons in bioassay water during exposure of cisco fry to varying dilutions of the water soluble fraction of Beaufort Sea crude oil.

Exposure of duckweed to mixtures of the Nektoralik WSF and Corexit dispersant failed to indicate significant toxicity over the 30-day experiment(Fig. 7) unless an actual slick was present. Although very little work has been done on aquatic flora(especially macrophytes) that work completed has shown a great diversity in species response(Snow,1979). Our work indicates duckweed to be an oil tolerant species. The growth inhibition that was observed when an oil slick was present is thought to represent a physical rather than chemical response as the same results were obtained using "Crisco" cooking oil.

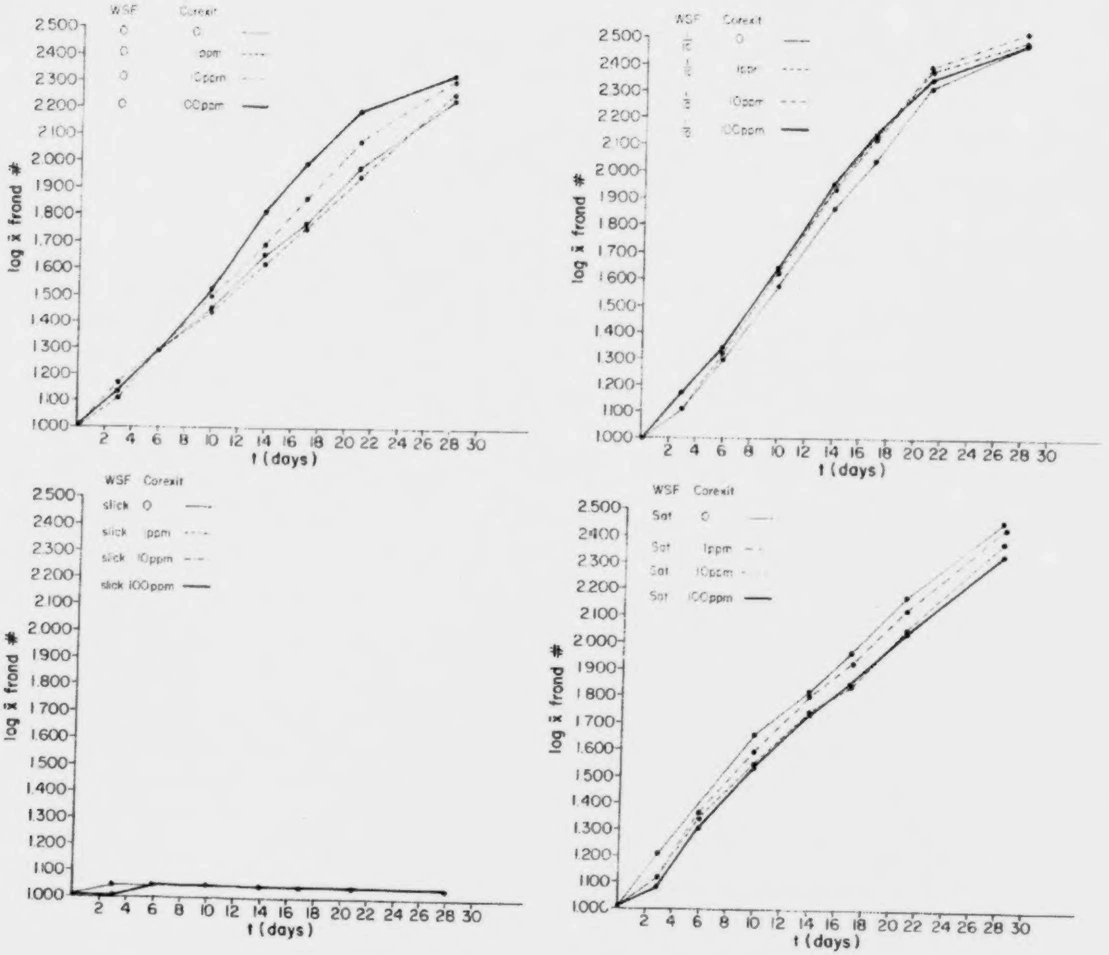

Fig 7 Growth curves for duckweed clones exposed to mixtures of the water soluble fraction(WSF)of Beaufort Sea crude oil and Corexit 9527 dispersant.

Table 1 Volitile hydrocarbons remaining in water(ppb)

(determined by headspace analysis for volatiles)

TIME AFTER ADDING COREXIT	0 PPM COREXIT	0.1 PPM COREXIT	1.0 PPM COREXIT	10 PPM COREXIT
0 Hr	1129	1195	1343	1128
2 Hr	955	1042	952	863
4 Hr	807	839	785	758
24 Hr	235	195	219	217
48 Hr	31	35	42	52
72 Hr	15	21	29	14
(determined by microextraction for nonvolatiles)				
0 Hr	1480	1085	1085	905
27 Hr	127	95	74	65
50 Hr	48	22	2	30

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INFLUENCE OF pH UPON ACUTE LETHALITY OF OLEIC AND LINOLEIC ACID TO RAINBOW TROUT

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[2]Satun Fisheries Station, LA-NGU, Satun, THAILAND

HRUDEY, Steve E. and Siri TOOKWINAS. 1982. Influence of pH upon acute lethality of oleic and linoleic acid to rainbow trout. Can. Tech. Rep. Fish Aquat. Sci. 1163.

Possible implications of sample pH upon regulatory bioassay monitoring of food processing industries were assessed by studying the acute lethality of two long chain unsaturated fatty acids: oleic and linoelic acid. While the sensitivity of contaminants such as cyanide and ammonia to pH influence on their toxicity is well known, such effects are not well documented for fatty acids. Each of the compounds was tested in replicate with Rainbow trout (Salmo gairdneri) at each of three nominal pH values (8.5, 7.0 and 6.5). Corresponding geometric mean LC50 value (5 replicates) decreased from 77 mg/L to 27 mg/L for oleic acid over this range. Geometric mean LC50 values (3 replicated) decreased from 9.5 to 5.2 mg/L for linoleic acid over the same pH range.

Key Words: pH, oleic acid, linoleic acid, rainbow trout, bioassay.

HRUDEY, Steve E. and Siri TOOKWINAS. 1982. Influence du pH sur le niveau de toxicité des acides oléique et linoléique sur la truite arc-en-ciel. Can. Tech. Rep. Fish Aquat. Sci. 1163.

Les applications possibles du pH dans les examens biologiques obligatoires dans les industries alimentaires ont été évaluées en étudiant la grande toxicité de deux acides gras non-saturés à longue chaîne, l'acide oléique et l'acide linoléique. Alors que la sensibilité de contaminants comme le cyanure et l'ammoniaque aux variations du pH en ce qui a trait à leur toxicité est bien connue, de tels effets sur les acides gras ne le sont pas. Chacun de ces composés a fait l'objet de plusieurs essais avec la truite arc-en-ciel (Salmo gairdneri) aux pH suivants: 8.5, 7, 6.5. Les valeurs géométriques moyennes correspondantes du CL50 (5 essais) ont diminué de 77 mg/L à 27 mg/L dans le cas de l'acide oléique; dans le cas de l'acide linoléique (3 essais), ces valeurs ont diminue de 9.5 à 5.2 mg/L.

INTRODUCTION

Industrial wastewater testing requirements for acute lethal toxicity have been widely adopted in guidelines associated with the Fisheries Act. Specified procedures call for testing of undiluted wastewater at the pH received. While the philosophy and purpose of such testing is open to debate, interpretation of results should be based upon some understanding of water chemistry interactions of relevant water contaminants. One factor of concern is the effect of sample pH (and dilution water pH where applicable) upon observed toxicity. Although the pH sensitivity of some contaminants (i.e., metals, cyanide, hydrogen sulphide and ammonia) is widely accepted, relatively few organic contaminants have been evaluated in this regard.

The long chain fatty acids represent a class of compounds commonly found in food processing and kraft pulping effluents. We selected two unsaturated fatty acids, oleic and linoleic acid, because:

1. they are usually significant components of wastewater lipids (Hrudey, 1981a);
2. they are two of the most water soluble long chain fatty acids; and
3. they have received relatively little attention as potential toxicants in industrial wastewaters.

Leach and Thakore (1973) reported an estimated lethal concentration to juvenile coho salmon (*Onchorhynchus kisutch*) of 8 to 9 mg/L for sodium linoleate and 12 to 13 mg/L for sodium oleate. These bioassays employed dechlorinated tapwater at pH 6.4 for dilution water. Curtis *et al.* (1977) reported 96 hr LC50 values using brine shrimp larvae (*Artemia salina*) of 0.033 mg/L for lineoleic acid and >0.087 mg/L of oleic acid.

MATERIALS AND METHODS

FISH HOLDING AND FEEDING CONDITIONS

Fish used in this study were rainbow trout (*Salmo gairdnerii* Richardson) fingerlings of either sex with weights and lengths as indicated in Table 1. The longest fish in each test was not more than 1.5 times the length of the shortest, as required by Standard Methods (APHA, 1975).

The fish were transferred from the Toxicology Laboratory, Environmental Protection Service, Edmonton to an acclimation room and were held at 9°C in a 1 000 L fiberglass holding tank at the Zoology Department, University of Alberta. They received a continuous flow of dechlorinated tap water (EDTA hardness = 116 mg/L as $CaCO_3$, conductivity = 225 µmho/cm, pH = 6.70, total alkalinity = 36 mg/L as $CaCO_3$, total residue = 180.8 mg/L and turbidity = 0.5 J.T.U.). Holding tanks were illuminated with fluorescent lights. These were electrically timed and switched to keep a photoperiod cycle with appropriate twilight periods similar to the local conditions.

The fish were fed once daily at the rate of about 3% of body weight. Fish were maintained in very good health and no significant mortality occurred. The fish were allowed to acclimate to these holding conditions for at least 2 weeks prior to experimentation. Feeding was stopped 48 hours before starting the experiment and the fish were transferred to the experimental rooms. Water temperature in the bioassay tanks was maintained constant at 12±1°C by room temperature control.

EXPERIMENTAL CONDITIONS

TEST TANKS

The test tanks were circular polyethylene tanks, 49 cm in diameter and 100 L in volume. Nineteen test tanks were placed in a temperature controlled room. Clean plastic bags containing 40 L of water sample were placed in the test tanks at the start of each experiment.

STOCK SOLUTIONS PREPARATION

Fatty acids themselves are only sparingly soluble in water. Therefore, a fresh stock solution of fatty acids (oleic and linoleic acids) was prepared by reacting the fatty acids with sodium hydroxide to produce sodium salts.

WATER PREPARATION

Normally, the hardness of dechlorinated Edmonton tap water would precipitate some of the fatty acids, resulting in a decrease in concentration and toxicity of the original fatty acids. Therefore, the dechlorinated tap water was diluted with an equal volume of distilled water to obtain the required dilution water for this study. The EDTA hardness as $CaCO_3$ was reduced from 116 mg/L to 60 mg/L.

EXPERIMENTAL PROCEDURES

ACUTE LETHALITY STUDIES

Rainbow trout fingerlings were tested with each fatty acid at 3 nominal levels of pH (6.5, 7.0 and 8.5). Five fatty acid concentrations were tested at each pH. Three control pH tanks, held at each of the nominal pH levels, were run at the same time. In total, 19 tanks were tested in each experiment. Rainbow trout fingerlings of either sex were equally distributed in a random manner into the 19 test tanks (10 fish per tank). Experiments with each fatty acid at each pH level were replicated (five replicated tests for oleic acid and two replicated tests for linoleic acid). All data from each fatty acid at each pH were pooled for statistical analysis. All tests were run for a 96-hour period.

Fish in each test tank were checked for mortality at 0.5, 1, 2, 4, 8, 24, 48, 72 and 96 hours. Dead fish were removed from the test tanks and time of death, weight and length of each fish were recorded. At the end of the 96-hour period, all fish that survived in each test tank were sacrificed and weight and length were recorded.

TEST WATER CHEMICAL CHARACTERISTICS

Water chemistry characteristics of test water in each test tank were checked for dissolved oxygen, pH and temperature at 12-hour intervals and EDTA hardness, alkalinity, ammonia-nitrogen and ether extractable matter (EEM) were checked at 24-hour intervals.

The procedures for water chemistry analysis were as follows:

1. Dissolved oxygen and temperature were measured with a dissolved oxygen meter (YSI Model 54A).
2. pH was measured with a pH meter (Hach portable Model 164000).
3. EDTA hardness was measured with Standard Method 309B, EDTA titrimetric method (APHA, 1975), using EDTA analytical reagent grade and eriochrome black T commerical indicator (Univer, Hach Chemical Co.).
4. Alkalinity was measured with Standard Method 403 (APHA, 1975), using phenolophthalein and mixed bromocresol green-methyl red indicators and 0.02N H_2SO_4 titrant.
5. Ammonia-nitrogen was measured with a Spectronic 20 (Bausch and Lomb), according to the nesslerization method 418B (APHA, 1975) using zinc sulfate solution, stabilizer reagent (rochelle salt solution), nessler reagent (HgI_2 + KI in KOH) and standard ammonia solution.
6. Specific fatty acids were identified by gas chromatography (Hewlett-Packard, 5736A), using the external standard technique with a flame ionization detector (FID) and esterification as per the method of Metcalfe et al. (1968). The gas chromatography conditions were as follows:
   - Column: Glass, 0.6 cm ID., 2 m long
   - Column Packing: 5% SP 2330, 100/120 Chromosorb W AW
   - Temperature: Oven 185°C
     Injection Inlet 250°C
     Detector 300°C
   - Gas Flow Rate: Carrier (N_2) 30 mL/min.
     H_2 30 psig.
     Air 27 psig.
   - Sample Size: 2μL
   - Attenuation: $2^4 = 16$
   - Chart Speed: 0.5 cm/min.
7. Ether extractable matter (EEM) was determined by continuous liquid-liquid extraction using petroleum ether as described by Hrudey (1981b). Due to the limited availability of extraction units, only four water samples and a control sample were collected for analysis each day. Of the four water samples, three were of the middle fatty acid concentrations at each pH level. These were collected each day. The fourth sample was collected from alternate concentrations on a pre-arranged schedule.

The gas chromatographic analysis was used to determine the purity of the test fatty acids (oleic = 87%, linoleic = 95%) and to monitor individual acid behaviour during tests. However, the basic quantitation of fatty acids was done by determining ether extractable matter (EEM) and using these figures as the effective fatty acid concentrations. The EEM data were used to generate a profile of fatty acid concentration stability over the duration of the bioassay (i.e., time to complete mortality or 96 hrs, whichever came first).

CALCULATION OF MEDIAN LETHAL CONCENTRATION

The median lethal concentration LC50 and its 95 percent confidence interval were calculated at 96-hour periods for each experiment by using the trimmed Spearman-Karber method (Hamilton et al., 1977) at 10% trim. The concentration used for each fatty acid was based upon the EEM value, interpolated between the start and the completion of the bioassay. This calculation was based on the stability profile mentioned above, since losses of fatty acids during 96 hours often exceeded 50%.

Comparison of the LC50 value for each pH level was done by two-way analysis of variance ($p < 0.05$) (Bennett and Franklin, 1954).

LC50 values obtained for individual tests on each acid were compared by an unpaired student t-test ($p < 0.05$) for each of the test pH series.

LC50 values from every replicated test of each acid were pooled for each of the test pH values and the geometric mean was determined.

pH CONTROL METHOD FOR DILUTION WATER

At pH 6.5: 50 mg/L of $NaHCO_3$ was added as a buffer and the pH was adjusted with 1N HCl at 12-hour intervals.

At pH 7.0: 100 mg/L of $NaHCO_3$ was added and the pH was adjusted with 1N NaOH at 12-hour intervals.

At pH 8.5: 100 mg/L of Na_2CO_3 was added and the pH was adjusted with 1N HCl and NaOH at 12-hour intervals.

Every test tank was aerated using an air compressor with an airstone in the center of the tank. Dissolved oxygen was checked every 12 hours and the air flow was adjusted for maintaining the dissolved oxygen in the tank above 9 mg/L.

RESULTS

OLEIC ACID

The relevant data for the 5 replicate bioassays performed on oleic acid (87% purity) are documented in Table 2. These data are summarized using the geometric mean LC50 values and corresponding 95% confidence limits and are plotted versus nominal test pH value in Figure 1. The geometric mean LC50 values decrease almost three-fold from 77 mg/L at pH 8.7 to 47 mg/L at pH 7.1 and 27 mg/L at pH 6.5.

The 96-hour LC50 values between the three nominal test pH levels are significantly different ($P < 0.05$, 2-way ANOVA). However, the differences between the replicate LC50 values at each test pH level are not significantly different ($P < 0.05$, 2-way ANOVA).

LINOLEIC ACID

The relevant bioassay data for the 2 replicate bioassays performed on linoleic acid (95% purity) are documented in Table 3. The geometric mean LC50 values are plotted vs nominal test pH values in Figure 2. These values decrease about two-fold from 9.5 mg/L at pH 8.8 through 7.1 mg/L at pH 7.1 to 5.2 at pH 6.5. The 96-hour LC50 values between the three nominal test pH levels are significantly different ($P < 0.05$, 2-way ANOVA). However, the differences between the replicate LC50 values at each test pH level are not significantly different ($P < 0.05$, 2-way ANOVA).

The 96-hour LC50 value obtained at each nominal pH value are significantly different between the two acids ($P < 0.05$, 2-way ANOVA).

DISCUSSION

The pH range of 6.5 to 8.8 is well within the reported range of pH tolerance of 4 to 10 (McLeay et al., 1979a). Hence, the finding of two- to three-fold changes in replicated LC50 values for a given pollutant is important.

Given the noted toxicity of many surfactant species, fatty acids might have been expected to show greater toxicity at higher pH. Certainly their surfactant properties are more pronounced in the anionic form than with the undissociated acid. Likewise, the solubility of these relatively low solubility compounds is much higher for the anionic form. The fact that the observed trend is in the opposite direction suggests that the surfactant character of fatty acids is not their sole, or even primary, toxic property.

Hunn and Allen (1974) found that passage of a toxicant across the gill membrane was predictable from the dissociation constant, pKa and lipid solubility of the chemical. For example, a weak acid was least toxic to fish at pH 9.5, a level where it would be completely dissociated. Similar results were reported by Marking (1975). He discussed the significance of the translocation of the un-ionized form of antimycin, a weak acid, across the gill tissue of fish. He found that the toxicity of antimycin was related to the concentration of un-ionized molecules and toxicity of antimycin decreased gradually from pH 6.5 to 8.5 with carp, green sunfish and bluegill sunfish.

Fatty acids in the ionized form, as they occur at higher pH, are not expected to pass through cell membranes as readily as the more lipid-soluble un-ionized form that exists at the lower pH. Therefore, the toxicity of these acids, if dependent upon transfer across the gill membrane, would decrease gradually with increasing pH of the test water.

The higher toxicity of fatty acid at the pH 6.5 test condition might also be due to a physical accumulation of the un-ionized form between the gill filaments leading to problems such as inhibited oxygen transfer.

Conn and Stumpf (1972) estimate the pKa of most fatty acids to be in the range of 4.75 to 5. Assuming a pKa value of 5.0, for oleic and linoleic acids the percentage dissociation can be calculated as a function of pH. This is illustrated in Figure 3. The analysis indicates that only about 3% of the total acid would be undissociated at pH 6.5. This value rises rapidly, however, to about 9% at a pH of 6. The acids would be essentially completely dissociated at the higher test pH values of 7.0 and 8.5.

Given the low fraction of undissociated acid involved, the results are remarkably similar to those reported by McLeay et al. (1979a, 1979b). They observed that toxicity of kraft pulp and paper mill effluents increased markedly as pH decreased from 8.5-9.5 to lower values. Specifically, the toxic resin acids, abietic and dehydroabietic acid, were evaluated in terms of their degree of dissociation at various pH values. Although these acids have pKa values of 7.15 and 7.25 respectively, the pH threshold at which they exhibited a marked rise in toxicity corresponded to only about 10% undissociated acid. Toxicity increased as the percentage of undissociated acid increased with decreasing pH. Hence, it appears, at least for resin acids, that even a small percentage of undissociated acid can cause a major increase in observed toxicity.

Alternately, the observed relationship between pH and LC50 value may represent separate stress mechanisms, one derived from the fatty acid and one derived from the decreasing pH. However, this explanation does not seem likely for pH values of 6.5 and above.

In any case, these results clearly demonstrate the need to consider test pH, even within an accepted nontoxic range when evaluating industrial effluents. It is likely that this principle will apply to a wide variety of organic pollutants which have not yet been evaluated for their pH dependence.

ACKNOWLEDGEMENTS

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Table 1. Weight and Length of Test Fish.

Toxicants	Test No.	Parameters	Means	Ranges	Standard Deviations
Oleic Acid	1	Weight*	0.80	0.29-2.10	0.37
		Length*	4.20	3.30-5.00	0.54
	2	Weight	0.97	0.30-3.19	0.50
		Length	4.57	3.50-7.00	0.67
	3	Weight	0.85	0.30-1.82	0.33
		Length	4.51	3.50-5.80	0.50
	4	Weight	0.52	0.18-1.25	0.23
		Length	3.93	2.90-5.30	0.52
	5	Weight	0.55	0.29-0.90	0.18
		Length	4.09	3.60-4.70	0.34
Average		Weight	1.22	0.18-3.19	0.56
		Length	4.91	2.90-6.00	0.76
Linoleic Acid	1	Weight	1.10	0.50-2.19	0.41
		Length	4.71	3.50-6.00	0.56
	2	Weight	1.63	0.52-2.70	0.96
		Length	5.08	3.60-6.20	0.52
Average		Weight	1.12	0.25-2.70	0.36
		Length	4.61	3.10-6.20	0.36

* unit, Weight = gram
Length = centimeter

Table 3. 96-Hour LC50 Values and 95% Confidence Intervals at 10% Trim for Linoleic Acid to Rainbow Trout Fingerlings.

	Test I			Test II		
	pH			pH		
	8.76 (8.4-9.0)	6.99 (6.7-7.5)	6.51 (6.5-6.6)	8.82 (8.4-9.0)	7.05 (6.75-7.60)	6.51 (6.5-6.6)
96-Hour LC50	10.6	8.2	5.7^{*2}	8.6	6.1	4.8
	(8.2-13.6)	(6.6-10.2)		(6.8-10.9)	(4.9-5.6)	(4.0-5.7)
Fish Length (cm)	4.57	4.81	4.76	5.08	5.02	5.13
Standard Deviation	0.54	0.58	0.49	0.57	0.45	0.53
Fish Weight (gm)	1.00	1.15	1.14	1.42	1.39	1.52
Standard Deviation	0.40	0.42	0.37	0.47	0.38	0.51
EDTA Hardness (mg/L as $CaCO_3$)	61.82	60.47	60.61	59.40	59.90	59.71
Standard Error of Mean	1.74	1.33	0.42	0.86	1.17	0.76
Dissolved Oxygen (mg/L)	9.53	9.49	9.61	9.67	9.54	9.48
Standard Error of Mean	0.11	0.06	0.13	0.22	0.12	0.07
Total Alkalinity (mg/L as $CaCO_3$)	114.34	96.03	50.56	113.08	95.54	57.72
Standard Error of Mean	2.94	3.25	0.63	1.32	4.37	0.90

*2 = 95% Confidence Interval Not Calculable.

Table 2. 96-Hour LC50 Values and 95% Confidence Intervals at 10% Trim for Oleic Acid to Rainbow Trout Fingerlings.

LC50 Concentration, mg/L as EEM

	Test I			Test II			Test III		
	pH			pH			pH		
	8.76 (8.5-9.0)	7.10 (6.6-7.5)	6.47 (6.4-6.6)	8.74 (8.40-9.0)	7.13 (6.75-7.50)	6.45 (6.4-6.5)	8.63 (8.3-9.0)	7.12 (6.8-7.6)	6.47 (6.45-6.50)
96-Hour LC50	60.4 (50.7-71.9)	48.6 (43.5-54.2)	22.7 (18.4-27.9)	75.8 (61.5-92.9)	44.7 (35.5-56.6)	23.4 (18.4-29.7)	74.6 (60.9-91.4)	48.6 (42.1-56.1)	26.9 (22.9-31.8)
Fish Length (cm)	4.26	4.33	4.30	4.52	4.90	4.50	4.64	4.38	4.38
Standard Deviation	0.41	0.62	0.61	0.55	0.79	0.62	0.52	0.54	0.44
Fish Weight (gm)	0.74	0.78	0.89	0.89	1.17	0.90	0.91	0.85	0.80
Standard Deviation	0.27	0.41	0.43	0.35	0.69	0.44	0.36	0.42	0.28
EDTA Hardness (mg/L as $CaCO_3$)	61.24	60.08	60.45	62.53	61.97	61.97	60.97	60.97	62.43
Standard Error of Mean	1.53	0.82	0.61	1.24	0.66	0.55	1.56	0.02	1.68
Dissolved Oxygen (mg/L)	9.21	9.25	9.32	9.52	9.53	9.64	9.49	9.51	9.53
Standard Error of Mean	0.06	0.08	0.03	0.11	0.13	0.22	0.22	0.29	0.32
Total Alkalinity (mg/L as $CaCO_3$)	125.26	85.33	61.06	111.42	90.58	57.23	117.24	99.19	60.03
Standard Error of Mean	1.32	0.66	0.72	5.73	6.39	1.82	4.08	3.88	1.14

	Test IV			Test V		
	pH			pH		
	8.46 (8.1-9.0)	7.09 (6.8-7.6)	6.52 (6.5-6.6)	8.76 (8.4-9.0)	7.11 (6.8-7.5)	6.51 (6.45-6.55)
96-Hours LC50	86.0 (73.1-101.0)	49.1*1 (48.0-51.9)	35.3 30.7-40.7)	93.1 (81.3-106.6)	42.6 (34.4-52.8)	29.8 (25.3-35.2)
Fish Length (cm)	3.75	3.95	3.75	4.00	4.32	3.90
Standard Deviation	0.43	0.57	0.52	0.35	0.29	0.26
Fish Weight (gm)	0.49	0.56	0.46	0.45	0.69	0.42
Standard Deviation	0.18	0.22	0.22	0.13	0.17	0.18
EDTA Hardness (mg/L as $CaCO_3$)	60.13	60.49	62.25	60.43	61.08	61.03
Standard Error of Mean	0.68	0.99	0.99	1.05	0.32	0.78
Dissolved Oxygen (mg/L)	9.37	9.41	9.42	9.51	9.56	9.53
Standard Error of Mean	0.11	0.09	0.11	0.06	0.04	0.05
Total Alkalinity (mg/L as $CaCO_3$)	121.74	99.72	59.23	114.06	97.48	58.95
Standard Error of Mean	0.90	1.16	0.59	0.56	1.04	0.32

*1 = at untrimmed calculable.

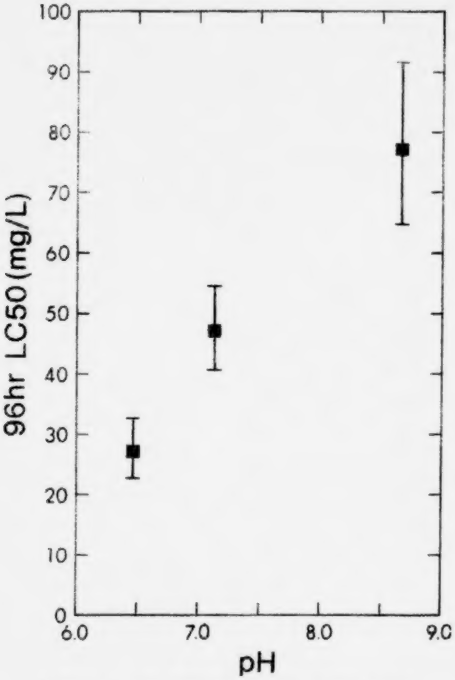

Figure 1 pH dependence of oleic acid geometric mean 96-hr LC50.

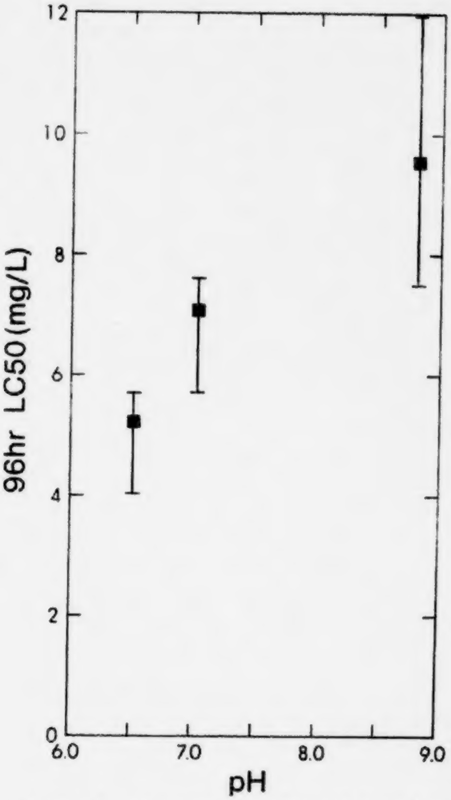

Figure 2 pH dependence of linoleic acid geometric mean 96-hr LC50.

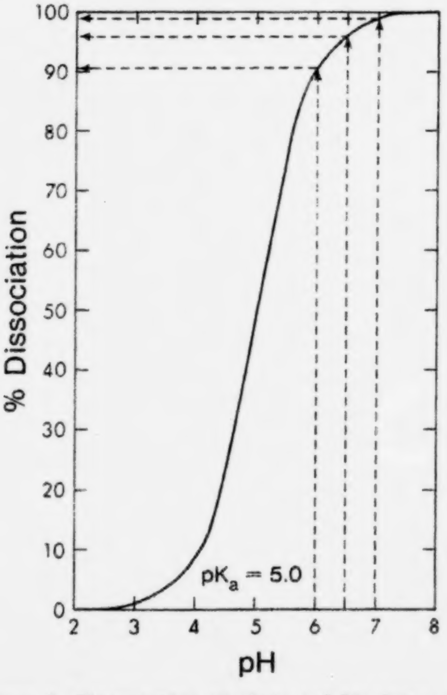

Figure 3 Dissociation of oleic and linoleic acids.

DYNAMICS OF CERTAIN REDOX REACTIONS OF SELENIUM IN FRESHWATER SEDIMENTS

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HUANG, P.M., D.W. OSCARSON, U.T. HAMMER, N.G. LIPINSKI, and W.K. LIAW. 1982. Dynamics of certain redox reactions of selenium in freshwater sediments. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The rates of certain redox reactions of Se (0,IV, VI) in two freshwater sediments (Katepwa and Buffalo Pound lakes) in Saskatchewan were studied. The appearance of Se(IV) in solution after a 72-hour reaction, when Se(VI) is the Se species initially added to the sediments, clearly indicates that the sediments have partially reduced Se(VI) to Se(IV). The concentrations of Se(IV) in solution after 7 weeks are 4-15% of the total Se remaining in solution. The rate of reduction of Se(VI) to Se(IV) appears to be related to E_h and reducing components of the sediments. In the absence of sediments, no detectable Se(VI) is reduced to Se(IV) within 7 weeks. The transformation of Se(IV) to Se(VI) in the sediments is not detectable within 7 weeks. Selenium (0) is autooxidized to Se(IV) and then partially absorbed by the sediments. The conversion of Se(0,VI) to Se(IV) in freshwater sediment systems merits close attention since Se species differ in their solubility and toxicity.

Key Words: Selenium, redox reaction, sediments, autooxidation.

HUANG, P.M., D.W. OSCARSON, U.T. HAMMER, N.G. LIPINSKI, and W.K. LIAW. 1982. Dynamique de certaines réactions d'oxydoréduction du sélénium dans les sédiments d'eau douce. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On étudie la rapidité avec laquelle se produisent certaines réactions d'oxydoréduction du Se(0,IV,VI) dans les sédiments de deux plans d'eau douce de Saskatchewan (les lacs Katepwa et Buffalo Pound). L'apparition de Se(IV) dans la solution d'expérimentation après un intervalle de 72 heures, alors que la substance ajoutée aux sédiments était du Se(VI), démontre clairement la réduction partielle du Se(VI) en Se(IV) par les sédiments. La concentration de Se(IV) dans la solution après sept semaines est de 4 p. 100 à 15 p. 100 de ce qui reste du Se en solution. Le taux de réduction de Se(VI) en Se (IV) semble lié au E_h des sédiments et à la présence d'autres réducteurs. On ne constate aucune réduction perceptible du Se(VI) en Se(IV) au cours des sept semaines d'expérimentation, lorsque la solution ne contient pas de sédiments. De même, il n'y a aucune transformation perceptible de Se(IV) en Se(VI) dans les sédiments au cours des sept semaines de tests. Le Se(0) s'oxyde pour former du Se(IV) et est ensuite partiellement absorbé par les sédiments. La conversion de Se(0,VI) en Se(IV) dans les sédiments d'eau douce mérite qu'on s'y attarde, car les diverses espèces de Se sont de solubilité et de toxicité differentes.

INTRODUCTION

Selenium is both essential and toxic to aquatic life and animal and human health (Demaya et al. 1979; Underwood, 1977; Fleming, 1980). It can enter freshwater environments through weathering (Bertine and Goldger, 1971) and anthropogenic activities such as the combustion of fossil fuels, mining, smelting, disposal of animal wastes and application of fungicides (Demayo et al., 1979). The pathways of Se entering the freshwater environments and subsequent movement to food chains are depicted in Fig.1. However, quantitative estimates for the amount of Se associated with each path (Fig.1) are almost non-existent.

The antagonistic and synergistic effects of Se on the toxicity of heavy metals, for example, Hg (Underwood, 1977; World Health Organization, 1978) deserve close attention in the area of environmental toxicology. Most studies with multicellular organisms have indicated that Hg and Se are less toxic when they are together (Reeder et al., 1979). Two possible mechanisms for Se protection against Hg toxicity have been proposed (Ohi et al., 1976): (1) Se may increase non-toxic binding sites which have a great affinity for heavy metals and hence divert them from usual targets, and (2) Se facilities conversion of methylmercury to a less toxic form. The contents of Se significantly vary with biological species. The levels of Se are generally higher in invertebrates and fish than in flora (authors' unpublished data).

The concentration of heavy metals in surface water is generally lower than the recommended limits (Fisheries and Environment Canada, 1977a, b). The bulk of these pollutants is immobolized in sediments. It is from sediments that much of these toxic pollutants could enter and concentrate in food chains (Fisheries and Environment Canada, 1979a). The bioavailability and toxicity of Se is highly dependent on its chemical forms (NAS, 1976; Fleming, 1980). Therefore, transformations of Se in freshwater environments have an important bearing on the movement of Se through the aquatic ecosystem to the subsequent food chains. Redox (oxidation-reduction) reactions are among important transformations of Se in the environment. The oxidation states of 0, +4, and +6 for Se are common in the environment (Demayo et al., 1979). Selenium in the +6 oxidation state (selenate) is stable in alkaline, oxidizing conditions. Selenate appears to be an important form of bioavailable Se. Selenium in the +4 oxidation state (selenite) adsorbs quite strongly on soil particles or precipitates as ferric selenite which has a very low suitability. Consequently, the transformation of selenate to selenite would lower the availability of Se to biota. Under more reducing conditions, very insoluble elemental Se could form and thus further decrease the availability of Se which enters the environment in the selenate or selenite form.

The objective of this study was to investigate rates of certain redox reactions of Se (0, IV, VI) in the sediments of the Katepwa and Buffalo Pound lakes in Saskatchewan.

MATERIALS AND METHODS

Reagants

Reagant grade Na_2SeO and Na_2SeO_4 were standardized gravimetrically (Erdey, 1965). Gray elemental Se was standardized by oxidizing 50 mg of Se(0) with 5 ml of 99% Br_2 (Uchida et al.,1980) in 500 ml of deionized-distilled water; the Se concentration was then determined fluorometrically as described below under "Determination of Se(IV) + Se(VI)".

The procedure given by Wilkie and Young (1970) was followed for the preparation of the 2, 3-diaminonaphthalene (DAN) complexing reagent.

Determination of Se(IV)

A 5 ml aliquot of a solution that contains Se was placed in a 50-ml glass Erlenmeyer flask. Twenty-five ml of deionized-distilled water and 5 ml of 0.06 M EDTA were added and the pH was adjusted to 1.8 ± 0.2 with 25% HCl. Two ml of DAN reagent was added, the flask was covered with Parafilm and the solution was heated at 60°C for 20 min in an oven. The solution was cooled in the dark for 45 min and then transferred to a 125-ml separatory funnel. Ten ml of n-hexane was added and the mixture shaken vigorously three times; the phases were allowed to separate for 4 min and then a portion of the hexane extract was collected in a fluorescence-measuring tube and the fluorescence determined by a Turner Model III fluorometer fitted with a NO. 7-60 (369 nm) primary filter and a NO.58 (525 nm) secondary filter. Selenium (VI) is not reduced by the above treatment and the DAN reagent reacts only with Se(IV) (Wilkie and Young, 1970). The concentration of Se(IV) can be determined in a solution containing both Se(IV) and Se(VI).

Determination of Se(IV) + Se(VI)

A 5 ml aliquot of a solution containing Se was placed in a round-bottom 25-ml boiling flask. Ten ml of deionized-distilled water and 1 ml of each of concentrated HCl (35-37%) and HBr (48%) were added. The flask was connected to a water-cooled condenser and heated at 90°C for 45 min in a water bath. This treatment quantitatively reduced Se(IV). The solution was cooled and transferred to a 50-ml glass Erlenmeyer flask; the boiling flask was rinsed with 10 ml of deionized-distilled water which was then transferred to the 50-ml Erlenmeyer. Five ml of the 0.06 M EDTA and 3 ml of 7 M NaCH were added and the pH was adjusted to 1.8 ± 0.2. The rest of the procedure was the same as that given for the determination of Se(IV). Since Se(IV) plus Se(VI) was determined by this procedure and Se(IV) was determined in the previous procedure, the amount of Se(VI) can be obtained by difference. The reagents used for the determination of total Se--HCl, HBr, and NaOH--contribute slightly to the fluorescence; this must be determined and accounted for.

The detection limit using this procedure is about 1 ng Se/ml.

Lake Sediments

Sediments from two freshwater lakes (Buffalo Pound and Katepwa) located in the upper Qu'Appelle River Basin in southern Saskatchewan, Canada, were selected for this study. The nature of the lakes and sediments are given elsewhere (Oscarson et al., 1981a). Bottom sediments were collected with an Ekman dredge. Several sediment samples from each lake were thoroughly mixed and combined to make a composite sample; the composite sample of each lake sediment was used for all experiments. The sediments were stored in sealed plastic bottles at 4°C.

Experimental of Redox Reactions of Se

Three grams (dry weight basis) of sediment was suspended in 300 ml of a solution that contained 100 ng/ml of Se(IV) or Se(VI) as Na_2SeO_3 or Na_2SeO_4 in 500-ml Erlenmeyer flasks. In other experiments 30 mg of 300-mesh Se(0) was added to 300 ml of deionized-distilled water or 3 g of sediment suspended in 300 ml of deionized-distilled water in a 500-ml Erlenmeyer flask. One hundred mg of birnessite, which was prepared by adding concentrated HCl to a boiling solution of $KMnO_4$ (McKenzie, 1971), was suspended in 300 ml of a solution that contained 100 ng Se(IV)/ml or mixed with 30 mg of 300-mesh Se(0) in 300 ml of deionized-distilled water and the pH was adjusted to 7.0.

For all experiments, the flasks containing the suspensions were stoppered and placed on an oscillating shaker in a water bath at $25^{0}+0.2^{0}$. At various time periods up to a maximum of 7 weeks, the suspensions were thoroughly mixed and a 15-ml aliquot of the suspension was withdrawn. This sample was centrifuged at 1000 x g for 30 min and the supernatant was filtered (0.45 μm pore size). Five ml of the filtrate was analyzed fluoremetrically for Se(IV) and 5 ml for Se(IV) plus Se(VI) as described above.

At each sampling period, the E_h (measured with a Pt electrode vs. an AgCl/Ag reference electrode in 4 M KCl) and pH of the suspensions were determined.

RESULTS AND DISCUSSIONS

The data presented in Table 1 show that satisfactory recovery of Se(IV) and Se(VI) added to the sediment extracts. This indicates the suitability of the proposed fluoremetric method for the quantitative differentiation of Se(IV) and Se(VI) in solution.

The appearance of Se(IV) in solution after a 72-hr reaction period, when Se(VI) is the Se species initially added to a suspension of the sediments (Fig.2), clearly indicates that the sediments have partially reduced Se(VI) to Se(IV); in the absence of sediment no detectable Se(VI) is reduced to Se(IV) within 7 weeks. The concentrations of Se(IV) in solution after 7 weeks is approximately 4% and 16% of the total Se in solution for the Buffalo Pound and Katepwa Lake sediment systems, respectively.

The conversion of Se (VI) to Se(IV) is a relatively slow process. The color of the sediment changes from a dark to a light brown 2 to 3 days after the sediment suspensions have been placed in flasks on an oscillating shaker at 25°C, indicating that the organic matter of the sediments is being oxidized. Selenium (VI) may serve as an electron in the oxidation of organic matter. The positive log K(W) value of the following reaction indicates that the reduction of Se(VI) to Se(IV) by organic matter, here CH_2O, is a thermodynamically favorable reaction,

$$2SeO_4^{-2} + CH_2O + 4H^+ \ (W)$$

$$= 2H_2SeO_4 + CO_2\ (g) + H_2O$$

$$\log K\ (W) = 54.7$$

The relevant half reactions for the organic material and Se were taken from Stumm and Morgan (1980) and Weast (1978), respectively; K (W) is the equilibrium constant for the redox reaction at pH and 25°C.

When Se(VI) is initially added to the sediment suspensions, the concentration of Se(IV) appearing in solution is greater in the Katepwa than it is in the Buffalo Pound Lake sediment system at any given time after 72 hr (Fig.2). This means that the Katepwa Lake sediment has a greater ability to reduce Se(VI) relative to the Buffalo Pound Lake sediment.

The Katepwa Lake sediment has a greater organic matter content than does the Buffalo Pound Lake sediment (Oscarson et al., 1981a); therefore, if organic matter is important in the reduction of Se(VI) the Katepwa Lake sediment would be expected to have the greater ability to reduce Se(VI). In addition, the E_n of the Katepwa Lake sediment system is lower, at least during the first part of the reaction period (during the latter part of the reaction periods, the E_n of the two sediment systems is similar). than that of the Buffalo Pound sediment system (Table 2). The lower initial E_n in the Katepwa sediment would also tend to produce the reduction of Se(VI) to Se(IV).

The sediments have a greater ability to sorb Se(IV) than Se(VI) (Figs. 2, 3, and 4).

Selenium(IV) can be adsorbed by allophanic clay (Rajan and Watkinson, 1976) and/or coprecipitates with iron oxides (Geering et al., 1968). Conversely, Se(VI) does not form stable sorption complexes or co-precipitates with Fe or Al oxides (Allaway et al., 1967). The sediments of the Buffalo Pound and Katepwa Lakes contain a series of hydrous oxides of Al, Fe, Mn, and Si (Oscarson et al., 1981a) which are apparently responsible for the sorption of Se. The sorption of Se(IV) by MnO_2 is illustrated in Fig.5.

When Se(IV) was the Se species initially added to the sediment suspension of the Katepwa Lake, the appearance of Se(VI) was not detectable within 7 weeks (Fig.3). On the contrary, when Se(IV) was added to the sediment suspension of the Buffalo Pound Lake the formation of Se(VI) in solution was observed (Fig.4). This is attributed to the higher initial E_n (Table 2) and lower organic matter content (Oscarson et al., 1981a) of the Buffalo Pound Lake sediment compared with the Katepwa Lake sediment.

When Se(0) is the Se species added initially to the Katepwa Lake sediment or birnessite (MnO_2) suspensions, the concentration of Se(IV) (no Se(VI) was detectable in these systems) in solutions is much less than when Se(0) is added to deionized-distilled water alone (Fig.6). Apparently the Se(0) is auto-oxidized to Se(IV) and then partially sorbed by the sediments.

Manganese oxides are present in the lake sediments used in this study (Oscarson et al., 1981a) and effective in converting As(III) to AS(V) (Oscarson et al., 1981b; Huang et al., 1982). However, the data obtained (Fig.6) indicate that MnO_2 does not catalyze the oxidation of Se(0) to Se(IV, VI) within 7 weeks despite the thermodynamic favorability of the following redox reactions:

$$Se + 2MnO_2 + 4H^+ = H_2SeO_3 = 2Mn^{+2} + H_2O \quad E^0 = 0.47$$

$$H_2SeO_3 + MnO_2 = SeO_4^{-2} + Mn^{+2} + H_2O \qquad E^0 = 0.06$$

This indicates that the kinetics of the oxidation of Se(0) by MnO_2 is relatively slow.

In conclusion, the data obtained indicate that the rates of redox reactions of Se (0, IV, and VI) in sediment-water systems significantly vary with E_n and components of sediments. The transformations of Se (0, IV, and VI) in freshwater sediment systems merit close attention since Se species differ in their solubility and toxicity.

SUMMARY

The rates of certain redox reactions of Se (0, IV, and VI) in two freshwater sediments (Katepwa and Buffalo Pound lakes) in Saskatchewan were studied. The appearance of Se(IV) in solution after a 72-hr reaction, when Se(VI) is the Se species initially added to the sediments, clearly indicates that the sediments have partially reduced Se(VI) to Se(IV). The concentrations of Se(IV) in solution after 7 weeks are 4-16% of the total Se remaining in solution. The rate of reduction of Se(VI) to Se(IV) appears to be related to E_n and reducing components of the sediments. In the absence of sediments, no detectable Se(VI) is reduced to Se(IV) within 7 weeks. The transformation of Se (IV) to Se(VI) in the Katepwa Lake sediments was not detectable but was clearly observed in the Buffalo Pound Lake sediments within 7 weeks. Selenium (0) is auto-oxidized to Se(IV) and then partially adsorbed by the sediments. The transformations of Se (0, IV, and VI) in freshwater sediment systems would affect its solubility, toxicity, and subsequent contamination to the food chain.

ACKNOWLEDGEMENTS

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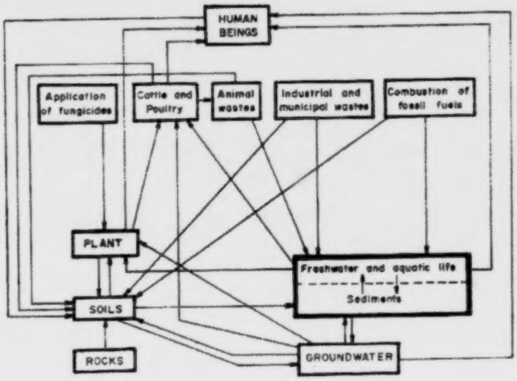
HUMAN BEINGS
Application of fungicides
Cattle and Poultry
Animal wastes
Industrial and municipal wastes
Combustion of fossil fuels
PLANT
SOILS
ROCKS
Freshwater and aquatic life
Sediments
GROUNDWATER

Fig. 1. Pathways for selenium entering freshwater environments and subsequent movement to food chains.

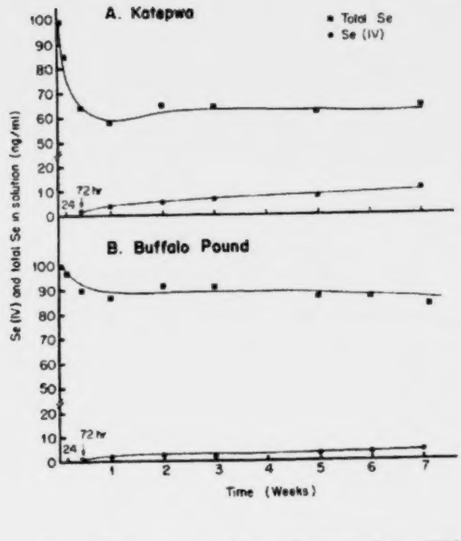

Fig. 2. The conversion of selenium (VI) to selenium (IV) by the Katepwa and Buffalo Pound Lake sediments. Total selenium is the sum of selenium (IV) and selenium (VI).

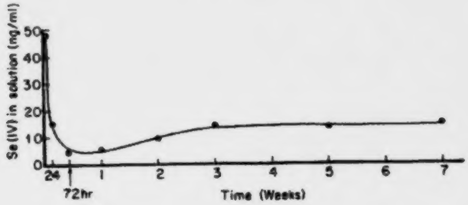

Figure 3. The sorption of selenium (IV) by the Katepwa Lake sediment. No detectable selenium (VI) was produced in solution.

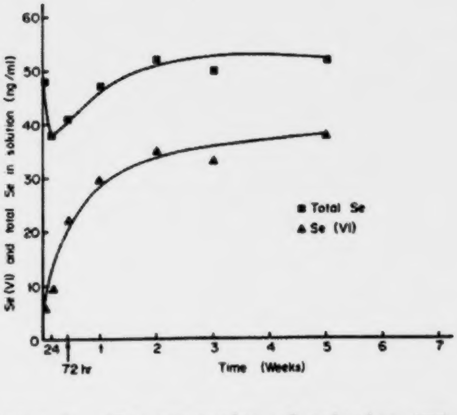

Figure 4. The conversion of selenium (IV) to selenium (VI) by the Buffalo Pound Lake sediments. Total selenium is the sum of selenium (IV) and selenium (VI).

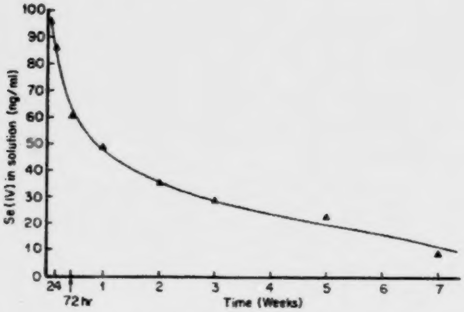

Figure 5. The sorption of selenium (IV) by birnessite (MnO_2). No detectable selenium (VI) was produced in solution.

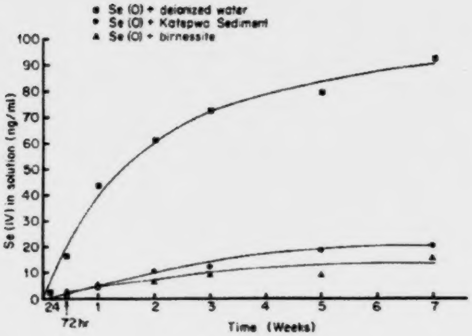

Figure 6. The auto-oxidation of selenium (O) to selenium (IV) and its subsequent sorption by the Katepwa Lake sediments and birnessite (MnO_2). No detectable selenium (VI) was produced in solution.

Table 1. Recovery of Se(IV) and Se(VI) added to extracts of the lake sediments.

ng Se added	ng Se recovered*	
	Katepwa	Buffalo Pound
I 250 Se(IV)	272±3**	250±0
250 Se(VI)	230±5	245±5
II 500 Se(IV)	468±12	497±13
15 Se(VI)	20±2	13±3
III 15 Se(IV)	12±2	17±4
500 Se(VI)	487±7	510±10

*The time period necessary to complete the experiment was less than 3 hr

**X±SD, n = 3

Table 2. pH and E_h values of the sediment suspension of the Katepwa and Buffalo Pound Lakes during the reaction period.

Reaction period	Katepwa		Buffalo Pound	
	pH[a]	E_h (mv)[a]	pH	E_h (mv)
3 hr	8.0	200	8.1	355
24 hr	7.6	260	8.1	365
72 hr	7.4	365	7.9	445
1 wk	7.2	370	7.7	525
2 wk	7.1	385	7.6	550
3 wk	7.0	400	7.7	520
5 wk	7.0	425	7.7	500
7 wk	7.1	440	7.5	443

[a] The precision of the pH and E_h measurements is ±0.1 and ±10 mv, respectively

THE EFFECTS OF GRADED LEVELS OF DIETARY COPPER ON THE GROWTH AND PHYSIOLOGICAL RESPONSE OF RAINBOW TROUT (*SALMO GAIRDNERI*)

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LANNO, R.P. and J.W. HILTON. 1982. The effects of graded levels of dietary copper on the growth and physiological response of rainbow trout (*Salmo gairdneri*). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The effect of geometrical increases of copper (Cu $SO_4.5H_2O$) from 0, 50 to 3200 mg.kg^{-1} in practical trout diets on the growth and physiological response of rainbow trout will be presented. The 'no effect' level of dietary copper on rainbow trout will be determined. The physiological effects of chronic dietary copper toxicity on blood parameters including hemoglobin, hematocrit, plasma copper and plasma glucose will be presented. Uptake and accumulation of copper in the carcass, liver and kidney in relation to dietary copper levels will be presented. Histopathology of the kidney, liver and intestine in relation to dietary copper will also be presented. (Supported by NSERC, OMAF and OMNR).

Key Words: Rainbow trout, *Salmo gairdneri*, copper, growth, chronic dietary copper, hemoglobin, hematocrit, plasma copper, uptake, accumulation.

LANNO, R.P. and J.W. HILTON. 1982. Effets de l'inclusion de diverses quantités de cuivre dans le régime alimentaire de la truite arc-en-ciel (*Salmo gairdneri*): sa croissance et ses réactions physiologiques. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'étude concerne les effets de l'augmentation géométrique --c'està-dire de 0 à 50 et 3 200 mg.kg^{-1} --du cuivre ($CuSO_4.5H_2O$) inclus dans le régime alimentaire de la truite sur la croissance et les réactions physiologiques de celle-ci. On y établit la quantité de cuivre qui peut-être incluse dans le régime alimentaire de la truite arc-en-ciel «sans effet». Sont également discutés les effets physiologiques d'un empoisonnement chronique dû au cuivre inclus dans le régime alimentaire sur les paramètres sanguins, notamment le niveau d'hémoglobine, l'hématocrite et la teneur en cuivre et en glucose du plasma. On signale en plus le rapport entre la proportion de cuivre dans les aliments et le taux d'absorption et d'accumulation de métal dans la carcasse, le foie et les reins. Enfin, on présente l'histopathologie des reins, du foie et des intestins en fonction de la dose de cuivre dans les aliments. (Avec l'aide financière du Conseil de recherches en sciences naturelles et en génie, du ministère de l'Agriculture et des Pêches de l'Ontario et du ministère des Ressources naturelles de l'Ontario).

INTRODUCTION

The toxicity level and physiological response of rainbow trout to water-borne copper (Cu) are well documented (Miller and Mackey, 1980; Howarth and Sprague, 1977). Rainbow trout exposed to Cu at 7 and 10 μg/L for 96 hours were more susceptible to infection by *Yersinia ruckeri* than control fish (Knittel, 1981). Exposure to copper at levels of 0.25 to 0.68 mg/L resulted in an initial cessation of feeding in rainbow trout, but ultimately adaptation to these levels of water-borne Cu occurred and feeding resumed (Lett et al., 1976). Rainbow trout exposed to water-borne Cu at 0.035 mg/L showed decreased liver ascorbic acid, lowered blood hemoglobin concentration and hematocrit levels and an increase in liver Cu levels compared with control trout (Yamamoto, 1981).

There have been very few studies on the response of rainbow trout to dietary Cu. Ogino and Yang (1980) found that a dietary Cu supplement of 0.7 mg/kg did not produce symptoms of Cu deficiency and suggested that a level of 3 mg Cu/kg of diet was an optimal dietary supplement. Murai et al. (1981) observed chronic dietary Cu toxicity in channel catfish fed semi-purified diets supplemented with Cu (as $CuSO_4$) at levels of 16 and 32 mg Cu/kg of diet. The chronic toxicity was characterized by reduced growth, poor feed conversion and a slight anemia when compared to catfish fed diets with Cu supplements of 8 mg Cu/kg of diet or less. In contrast, Knox et al. (1982), attempting to determine the effect of dietary Cu and the Cu:Zn ratio on rainbow trout, found no dietary Cu:Zn antagonism and no effect on growth when fish were fed a dietary Cu supplement of 150 mg/kg of semi-purified diet. Although Murai et al. (1981) observed toxicity in channel catfish at a level of 16 mg/kg, no dietary Cu toxicity was observed in rainbow trout at a level of 150 mg Cu/kg diet (Knox et al., 1982). Hence, dietary Cu tolerance in rainbow trout appears to be higher than for channel catfish and to exceed 150 mg/kg diet. The purpose of this preliminary study was to determine the toxic level of dietary Cu in rainbow trout, and to determine the effect of dietary Cu toxicity on growth, certain blood parameters and Cu uptake and accumulation in the rainbow trout.

MATERIALS AND METHODS

Experimental Design and Environmental Parameters

Rainbow trout fingerlings were fed practical trout diets supplemented with Cu (as $CuSO_4 \cdot 5H_2O$) at levels to provide from 0 to 3200 mg Cu/kg diet for a period of 8 weeks. The 8 diets were randomized to eight 100 L tanks as a completely randomized design. Flow rate into the tanks was 3.5 to 4.0 L/min and the water temperature was maintained at 14.98 ± 0.11^oC (mean = SD, n=56). Water quality parameters were as follows: dissolved oxygen-6.3 to 8.1 mg/L; hardness (EDTA) - 140 mg/L $CaCO_3$; pH - 7.8; NH_3-4.4 μg/L; Cu - <20 μg/L; An - 44 μg/L. After 8 weeks, weight gain, food conversion efficiency and other physiological parameters were determined in the trout.

Diet Preparation

Eight practical diets were formulated with geometrically increasing levels of Cu supplementation (as $CuSO_4 \cdot 5H_2O$) from 0 to 3200 mg/kg of Cu (Table 1). Diets were processed by steam pelleting. Copper content of each diet was determined by atomic absorption spectrophotometry (Perkin-Elmer Model 372, Norwalk, CT.) using an oxy-acetylene flame. Accurately weighed (0.1 to 1.0 g) samples of diet were dry-ashed in a muffle furnace for 4 hours at 600^oC. The ashed diet was taken up quantitatively with 3N HCl and diluted to 25 mL with double-distilled, de-ionized water. Standards were prepared using Fisher Certified Atomic Absorption Standard Stock Solution (1,000 mg/: ± 1%). All glassware and crucibles were soaked overnight in concentrated nitric acid.

Supply and Maintenance of Fish

Rainbow trout (*Salmo gairdneri* Richardson) were obtained from Goossen's Trout Farm Ltd., Otterville, Ontario. After a two-week acclimation period, 200 fish per tank (mean weight 3.80 ± 0.026 g/fish) were randomly counted into eight 100 L grey fiberglass tanks with water maintained at a temperature of 14.98 ± 0.11^oC. The tanks were serated and were kept as a flow through system with a water inflow rate of 3.5 to 4.0 L/min, using a well water - City of Guelph water supply mixture. The test diets were fed to single tanks of 200 fish for 8 weeks. Fish were fed 4 times per day and the amount of food per feeding was determined as described by Hilton and Slinger (1981).

Growth and Biochemical Assays

Body weights, feed:gain ratios, and total mortalities were determined after each 28-day period. After 8 weeks, fish were randomly selected from each tank and killed with an overdose of tricaine methanesulphonate (Syndel Laboratories Ltd., Vancouver, B.C.). Livers and head kidneys were excised, quick-frozen with liquid nitrogen, and stored at -20^oC until assayed for ascorbic acid content. Livers and kidney samples to be used for Cu and Zn determination were excised, freeze-dried, and stored at -20^oC until these determinations could be performed.

Blood for hematological analysis was collected from six to eight fish (fasted 18 hr) per tank per analysis, in heparinized centrifuge tubes, following caudal peduncle severance. Blood samples were immediately centrifuged at 3500 x g for 5 min, and the plasma stored at -20°C. Plasma samples to be analyzed for plasma glucose content were taken from the fish no longer than 120 seconds after the cessation of opercular movement when subjected to an overdose of tricaine methanesulphonate. Plasma glucose was determined by the glucose oxidase method (Glucose GOD-PAP Kit, Mannheim-Boehringer Canada Ltd., Dorval, P.Q.). Total plasma Cu was analyzed by discrete nebulization atomic absorption spectroscopy (Makino and Takahara, 1981). Hemoglobin was determined by the cyanmethmeglobin method (UNOPETTE TEST 5859, BECTON-DICKINSON, Rutherford, New Jersey). Hematocrit values were obtained using 20 μL heparinized capillary tubes.

For Cu and Zn analysis freeze-dried liver and kidney samples (0.5 to 1 g) were placed in 20 mL conc. HNO_3 and brought to boil for approximately 5 min. The solution was cooled and 5 mL of $HClO_4$ was added for further digestion until it cooled and made up to volume (25 mL) with double-distilled, de-ionized water. The samples were analyzed by atomic spectroscopy. The ascorbic acid concentration of fish tissues was determined by the method of Aeschbacher and Brown (1972). Weighed tissue samples (0.5 g liver; 0.1 g kidney) were homogenized in 5.0 mL of 6% trichloroacetic acid (TCA, w/v). The homogenized sample was then centrifuged for approximately 5 min at 70,000 g. One mL of the supernatent was then mixed in another test tube with 3 mL of TCA, to which was then added 1 mL of chloramine T:buffer solution. The solution was mixed and allowed to stand for 10 min. Two drops of thiourea and 1 mL of 2.4-dinitrophenylhydrazine reagent were then added and the sample was mixed well and incubated for 3 hr at 37°C. After the incubation period, the samples were removed from the incubator, rapidly cooled in an ice bath, and 5 mL of 85% H_2SO_4 (v/v) was slowly added. The test tubes were shaken and allowed to stand for 5 to 10 min. Optical density of the solutions was measured at 540 nm in a spectrophotometer. Tissue concentrations were then determined by reference to a standard curve prepared using ascorbic acid U.S.P. (Hoffman-La Roche, Vaudreuil, P.Q.).

Statistical Analysis

Experimental results were subjected to either linear regression or analysis of variance. Differences between treatment means were determined at the 5% probability level using Tukey's procedure as described in Steel and Torrie (1980).

RESULTS

Growth Study

After 8 weeks, there was a noticeable reduction in weight gain and feed intake concomitant with an increase in feed:gain values and mortalities in fish fed diets containing ≥ 730 mg Cu/kg diet (Table 2). A pronounced refusal of food and a marked increase in mortalities was noted in groups fed 1585 or 3088 mg Cu/kg diet when compared to groups of fish consuming diets with ≤ 730 mg Cu/kg of diet.

Blood Parameters

Hematocrit and hemoglobin levels varied little in fish fed diets 1 through 7, and were significantly lower only in fish fed diet 8. Hematocrit and hemoglobin concentrations were both at their highest values in fish fed diet 3 (83 mg Cu/kg). Mean plasma glucose values fluctuated significantly between diets but no trend in serum glucose concentrations was apparent. There were no significant differences in total plasma Cu levels among treatments.

Tissue Mineral Content

Liver and kidney analysis revealed that the Cu content of both tissues increased with increasing levels of dietary Cu (Table 4). Liver, but not kidney Zn levels also appeared to increase with increases in dietary Cu concentration. When livers were excised from fish fed the 730 mg Cu/kg diet, or more, large areas of pale tissue were noted on the liver. These lesions occurred in approximately 80% of the fish observed.

Mean ascorbic acid levels fluctuated significantly among treatments (Table 5) but there appeared to be no definite relationship of tissue ascorbic acid to increasing levels of dietary Cu.

DISCUSSION

Rainbow trout reared on diets containing >730 mg Cu/kg diet (Table 1, groups 6, 7, and 8) exhibited food avoidance and refusal, reduced food consumption and the appearance of gross lesions on the liver. The food refusal observed consisted of fish either mouthing the pellets of feed and subsequently expelling them or simply swirling at the pellets and not mouthing them at all. Signs of food refusal were most pronounced in fish fed diets containing 1585 or 3088 mg Cu/kg diet. These signs coupled with the significantly lower body weights, higher feed:gain ratios and higher mortality rates (Table 2) indicate that dietary Cu toxicity occurred in the range of 287 to 730 mg Cu/kg diet.

Hemoglobin concentrations and hematocrits fluctuated significantly among treatments, but showed no definite trends and remained within the "normal" range of literature values for rainbow trout (McCarthy et al., 1975; Wedemeyer and Nelson, 1975). The depressed hemoglobin and hematocrit values observed in fish fed the diet containing 3088 mg Cu/kg of diet may be an effect of inanition rather than of elevated dietary Cu. Knox et al. (1982) observed a decrease in hematocrit and hemoglobin concentrations in rainbow trout fed diets containing 150 mg Cu/kg of diet as compared to fish fed a supplement of 15 mg Cu/kg, but "depressed" values were well within the "normal" range.

Plasma Cu levels did not change in response to increasing levels of dietary Cu. This is in agreement with the results of Knox et al. (1982) and Murai et al. (1981) working with rainbow trout and channel catfish, respectively. Liver and kidney Cu levels appear related directly to dietary Cu intake. As dietary Cu levels increased, there also appeared to be a concomitant increase in hepatic Zn levels. Although Knox et al. (1982) found that increasing dietary Cu levels increased the amount of Cu present in the liver, no change in liver Zn levels was noted. It is possible that higher levels of dietary Cu than those used by Knox et al (>150 mg Cu/kg diet) may be required to affect the absorption and transport of zinc in the rainbow trout.

The accumulation of Cu in the liver may be indicative of the onset of Cu toxicosis. Prior to the manifestation of overt symptoms of Cu toxicosis in sheep (Gopinath et al., 1974) and in swine (De Goey et al., 1971), a large increase in hepatic Cu levels was noted. A similar situation may occur in rainbow trout as no signs of Cu toxicosis such as depressed growth and higher feed: gain values were observed until Cu had accumulated to a level of approximately 880 μg/g (dry weight basis) in the liver of fish fed diets containing >730 mg/kg Cu.

Plasma glucose levels were variable and showed no significant trends. Plasma glucose levels were in the high "normal" range for rainbow trout (McCarthy et al., 1975; Wedemeyer and Nelson, 1975) and this may be attributable to the stress of sampling.

Ascorbic acid concentration in liver and head kidney of rainbow trout seemed unaffected by increase in dietary Cu levels. This is in direct contrast to work done by Yamamoto et al. (1981) who noted a marked decrease in hepatic ascorbic acid stores in rainbow trout exposed to a 0.035 mg/L Cu solution for 18 weeks when compared to controls. Differences in ascorbic acid:Cu interaction may be to the duration of the experiment (18 weeks vs. 8 weeks) or simply due to differences in the effects of dietary and waterborne modes of administration of Cu.

NRC (1980) suggests that a significant time perid (weeks to months) is usually required for the development of the signs of chronic dietary Cu toxicosis, and that their ultimate expression is so rapid that the fatal course appears to be caused by an acute process. A subsequent experiment is therefore required to determine the effects of a greater duration of dietary Cu exposure on rainbow trout than used herein.

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TABLE 1: Composition of the basal test diet and levels of dietary copper supplementation.

Basal Diet

Ingredient	%
Capelin Meal	38
Soybean Meal	25
Wheat Middlings	22
Vitamin PreMix[a]	2
Mineral Pre-Mix[a] (Cu-free)	1
Fish Oil	12

Dietary Copper[b] Content[c]

Diet	Nominal	Determined[d]
1	0	9.0±.14
2	50	37±1
3	100	83±1.3
4	200	171±15
5	400	287±16
6	800	730±209
7	1600	1585±198
8	3200	3088±650

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[a] Vitamin and Mineral Pre-mix as given in Cho et. al. (1976), ($CuSO_4$ not added to Mineral Pre-mix)

[b] as $CuSO_4 \cdot 5H_2O$

[c] mg/kg, wet weight

[d] Mean ± SD, n=3

TABLE 2: Growth performance of rainbow trout after 8 weeks on the test diets at 15°C.

Diet	Weight gain (kg/100 Fish)[ac]	Food intake (kg/8 wks.)[c]	Food:gain[a] ratio	Mortalities[b]
1	2.53	4.768	0.97	6.4
2	2.43	4.485	0.96	9.2
3	2.50	4.671	0.97	8.6
4	2.54	4.563	0.95	12.3
5	2.52	4.386	0.92	12.3
6	1.12	2.929	1.36	9.3
7	0.37	1.027	1.53	33.8
8	0.18	0.667	2.20	46.7

[a] Initial body weight 3.80 ± .08 g/fish/tank

[b] Number of mortalities/10,000 fish days

[c] Estimate on a per tank basis

TABLE 3: Blood parameters[2] in rainbow trout fed test diets for 8 weeks. (Values in columns with the same superscript are not significantly different).

Diet	Hematocrit	Hemoglobin (g/dL)	Serum Glucose (mg/dL)	Plasma Cu (µg/mL)
1	43.8 ± 2.0^{ab}	$7.6 \pm .96^{abc}$	99.3 ± 8.9^{c}	$1.2 \pm .19^{a}$
2	41.2 ± 3.8^{ab}	$7.3 \pm .90^{ab}$	98.4 ± 15.4^{c}	$0.90 \pm .20$
3	45.6 ± 5.7^{a}	$9.2 \pm .92^{cd}$	101.8 ± 12.8^{c}	$1.03 \pm .12^{a}$
4	43.1 ± 3.3^{ab}	$9.0 \pm .92^{cd}$	78.8 ± 6.4^{ab}	$1.1 \pm .19^{a}$
5	42.0 ± 3.9^{ab}	$8.0 \pm .91^{abcd}$	65.9 ± 5.8^{a}	$1.1 \pm .09^{a}$
6	44.5 ± 3.2^{ab}	8.7 ± 1.0^{bc}	88.3 ± 9.5^{bc}	$1.1 \pm .14^{a}$
7	40.1 ± 2.8^{b}	$7.5 \pm .48^{abc}$	ND[1]	ND
8	32.9 ± 2.9^{c}	$7.0 \pm .67^{a}$	ND	ND

[1] Not determined

[2] Mean ± SD (Hematocrit and Hemoglobin, n=6; Serum Glucose and Plasma Cu, n=3).

TABLE 4: Copper and zinc content[a] of liver and kidney[c] of rainbow trout after 8 weeks on the test diets.

Diet	Liver		Kidney	
	Cu	Zn	Cu	Zn
1	127.3	62.8	4.95	94.6
2	175.0	59.8	11.5	86.2
3	247.8	60.3	10.6	84.6
4	350.8	63.3	13.8	89.1
5	509.0	69.8	22.8	90.6
6	886.0	75.3	76.2	95.5
7	1282.0	83.8	ND[b]	ND
8	3183.0	107.3	ND	ND

[a] µg/g dry weight.

[b] Not determined

[c] Single determination performed on a pooled sample of liver or kidney from 20-25 fish.

TABLE 5. Ascorbic acid content (wet weight) of rainbow trout liver[2] and head kidney[2] after 8 weeks on test diets.

Diet	Liver[1]	Head Kidney[1]
	(µg/g±S.D.)	
1	148.0 ± 9.2^{a}	166.0 ± 18.4^{ab}
2	140.1 ± 7.7^{a}	145.8 ± 7.8^{a}
3	144.4 ± 8.7^{a}	160.4 ± 9.9^{a}
4	167.7 ± 35.9^{a}	208.6 ± 30.4^{b}
5	171.5 ± 12.5^{a}	167.7 ± 12.9^{ab}
6	129.6 ± 6.7^{a}	145.7 ± 13.3^{a}

[1] Values in columns with the same superscript are not significantly different.

[2] 3 replications on a pooled sample of livers and head kidneys from 20-30 fish.

THE TOXICITY OF WATERS PRODUCED DURING IN-SITU RECOVERY OF OIL FROM THE ATHABASCA OIL SANDS AS DETERMINED BY THE MICROTOX BACTERIAL SYSTEM

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MACLEAN, Alice and Eric PEAKE. 1982. The toxicity of waters produced during in-situ recovery of oil from the Athabasca oil sands as determined by the Microtox bacterial system. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Waters produced during in-situ recovery of oil by the steam stimulation and the COFCAW (combination of forward combustion and water flooding)processes were found to be toxic in the Microtox bioluminescent assay. EC50 values, the effective concentration of toxicant causing a 50% decrease in the light output of a photoluminescent bacteria, ranged from 0.3 to 11.4 per cent. Volatile organic compounds were analyzed by combined gas chromatography-mass spectrometry using a purge and trap method. Extractable organic compounds were also analyzed by combined gas chromatography-mass spectrometry after separation on ion exchange resins or fractionation by acid-base partition. The toxicity of the waters was caused partly by volatile organic compounds, primarily alkyl substituted benzenes, and partly by extractable organic compounds including phenols, organic acids and hydrocarbons, with no single class of compounds solely responsible for the observed toxicity.

Key Words: Microtox, toxicity, benzenes, phenols, organic acids, hydrocarbons.

MACLEAN, Alice and Eric PEAKE. 1982. Toxicité des eaux produites pendant la récupération in situ du pétrole contenu dans les sables bitumineux de l'Athabasca, d'après la méthode d'expérimentation bactérienne Microtox. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'analyse de bioluminescence Microtox révèle que les eaux produites pendant la récupération in situ du pétrole, soit par injection de vapeur, soit par l'application du procédé COFCAW (combustion co-courant avec injection d'eau), sont toxiques. La concentration efficace de substance toxique causant une baisse de 50 p. 100 de la lumière produite par des bactéries photoluminescentes, ou CI_{50}, relevée au cours de l'expérience, varie de 0,30 p. 100 à 11,4 p. 100. Les composés organiques volatils sont analysés par chromatographie en phase gazeuse et par spectrométrie de masse, après leur concentration par épuration et par séparation. Les composés organiques extractibles sont analysés de la même manière, après leur répartition sur des résines ioniques ou leur fractionnement au moyen de la séparation en acides et en bases. Il ressort de cette analyse que la toxicité des eaux est causée en partie par les composés organiques volatils, les benzènes alkylés surtout, et en partie par les composés organiques extractibles, notamment les phénols, les acides organiques et les hydrocarbures, aucune de ces catégories n'étant toutefois la seule cause de la toxicité relevée.

THE TOXICITY OF WATERS PRODUCED DURING IN-SITU RECOVERY OF OIL FROM THE ATHABASCA OIL SANDS AS DETERMINED BY THE MICROTOX BACTERIAL SYSTEM

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ABSTRACT

Waters produced during in-situ recovery of oil by the steam stimulation and the COFCAW (combination of forward combustion and water flooding) processes were found to be toxic in the Microtox bioluminescent assay. EC_{50} values, the effective concentration of toxicant causing a 50% decrease in the light output of a photoluminescent bacteria, ranged from 0.30 to 11.4 per cent. Volatile organic compounds were analyzed by combined gas chromatography-mass spectrometry using a purge and trap method. Extractable organic compounds were also analyzed by combined gas chromatography-mass spectrometry after separation on ion exchange resins or fractionation by acid-base partition. The toxicity of the waters was caused partly by volatile organic compounds, primarily alkyl substituted benzenes, and partly by extractable organic compounds including phenols, organic acids and hydrocarbons, with no single class of compounds solely responsible for the observed toxicity.

INTRODUCTION

The toxicity to aquatic organisms of waters produced during the recovery of oil from the Athabasca oil sands by in-situ recovery processes is of environmental concern. Similarly the toxicity of waters which may contact produced hydrocarbons or the upgraded synthetic crude oil is of importance, for although the production and upgrading of oil may be rigorously controlled, spills will inevitably take place during production and transportation of upgraded oil. Such spills may directly affect surface waters and eventually affect groundwaters through the leaching of water soluble components from contaminated soil.

The objectives of this study were to determine (1) the toxicity of waters coproduced with hydrocarbons during in-situ recovery of oil by the COFCAW (combination of forward combustion and water flooding) and steam stimulation processes, (2) the toxicity of waters contaminated with upgraded synthetic crude oil, and (3) the classes of chemical compounds responsible for this toxicity.

EXPERIMENTAL

The Microtox bioassay system was used to determine the toxicity of produced waters (a) as received, (b) after purging with nitrogen, (c) after chemical fractionation into acidic, phenolic, basic and neutral compounds, and (d) after extraction of organic compounds. Chemical fractionations were carried out on ion exchange resins and solvent extractions were conducted under acidic, neutral, and basic conditions. Chemical components of the volatile and extractable organic fractions were identified by combined gas chromatography-mass spectrometry.

SAMPLE DESCRIPTION

Waters were from the Gregoire Lake pilot project located about 40 km southeast of Fort McMurray, Alberta. This pilot project was designed for the extraction of deep oil from the Athabasca oil sands by the COFCAW process which consists of ignition of the formation, injection of air to control combustion, and injection of water to create a steam pressure. A portion of the bitumen is combusted and some is thermally or pyrolytically altered.

Initially the Gregoire Lake project was to consist of nine injection wells and sixteen production wells with a spacing of one production well per hectare. Subsequently the spacing of wells was modified by the addition of satellite wells located in each case about 8 meters from the production wells in the direction of the injection wells. Not all wells within the pilot project produced by the COFCAW process; some produced by steam stimulation alone.

Communication among injection and neighbouring production wells was not always well established or defined; thus produced waters may have been derived directly from the COFCAW process, from a combination of the COFCAW process and steam stimulation or from steam stimulation alone. Some original connate water may also have been produced.

Fluids from the Gregoire Lake pilot project were sampled on February 19, March 19, May 14 and June 10, 1981. A total of five wells were sampled with produced water and oil-water emulsion collected in 20 litre glass containers and in 50 mL culture tubes tightly closed with teflon lined screw caps. Not all wells were producing during each sampling period. A list of samples is given in Table 1.

The well designated GP-26, when sampled in May, provided a good example of COFCAW produced fluids. Wells GP-31, sampled in February and March, and GP-19, sampled in May and June, produced primarily by steam stimulation. Wells GP-20 and GP-21 produced a mixture of injected water and steam. In most cases samples were obtained from the test separator, but when such a sample was not available group separator samples were taken. Near-surface groundwater which was injected during the COFCAW operations was also sampled.

MICROTOX BIOASSAY OF TOXICITY

In the Microtox system a marine luminescent bacteria was used as the bioassay organism. Osmotic protection of the bacteria is provided by the addition of sodium chloride to the test solutions. The bacteria display good light stability in the presence of 2% sodium chloride with all components of the test being adjusted to this salinity.

Lyophilized luminescent bacteria were hydrated before each test with 1.0 mL of distilled water precooled to 3.0°C ± 1°C. Five to seven pairs of cuvettes were placed in an incubator block controlled at 15°C. To one cuvette of each pair, the test cuvette, was added 500 uL of Microtox diluent (purified water containing 2% NaCl). The second cuvette of each set received 1.0 mL of an appropriately diluted sample with the exception of one cuvette which contained Microtox diluent which was used to monitor any time dependent drift in light output.

Ten uL of the solution containing rehydrated bacteria was added to each of the cooled test cuvettes and allowed to stabilize for 15 minutes. Initial light readings were then recorded for each cell suspension using a a Microtox analyzer. Five hundred uL of the solution from the blank cuvette and from each of the sample cuvettes was transferred to the appropriate cell suspension. Light output from each cuvette was recorded after 5-minute and 15-minute reaction periods.

The decrease in light, after correction for any time dependent drift, versus the concentration of the sample was used to calculate the toxicity of the sample.

CHEMICAL ANALYSES

Total Organic Carbon (TOC)

Samples collected in 50 mL culture tubes were analyzed for total organic carbon with a Dohrmann Envirotech organic analyzer. The sample was shaken prior to analysis; thus the TOC values represent both dissolved organic compounds and compounds associated with suspended matter. The method also gave a value for "volatile organic carbon" representing compounds boiling up to about 150°C.

Volatile organic compounds

Volatile organic compounds were analyzed by combined gas chromatography-mass spectrometry after concentration by a modified purge and trap method (Federal Register 1979). From 0.5 to 5.0 mL of sample was placed in a 13 x 100 mm culture tube and the volume brought to 5 mL by the addition of distilled water as necessary. The tube was heated to 45 C in a water bath and helium passed through the sample via a length of fused silica capillary tubing at the rate of 50 mL per minute. The purged hydrocarbons were trapped in a 40 mm x 10 mm glass column filled with Tenax GC resin. The hydrocarbons were removed from the Tenax resin by heating to about 250°C with helium flowing at 50 mL per minute and were trapped in a length of fused silica tubing at liquid nitrogen temperature. The hydrocarbons were then flashed into the injection port of a combined gas chromatograph mass spectrometer (GC-MS) and analyzed. The GC-MS system used was a Finnigan Model 4021 equipped with an INCOS data system, Wanco 10 megabite disc drive, and Perkin Elmer 9 track tape drive. The GC column was a 30 m x 0.25 mm fused silica column coated with SE54.

Extractable organic compounds

Water samples were extracted in the 20 litre bottles used for sample collection. The neutral and acidic organic components were extracted by first acidifying the sample to pH 2 with concentrated hydrochloric acid and then mixing for a period of 6-18 hours with 200 mL of benzene using a magnetic stirrer. After allowing the aqueous and solvent phases to separate, the benzene and any emulsion were removed. Four to 5 extractions were made with 200 mL of benzene per extraction.

Following extraction of the acidic and neutral compounds, the water sample was adjusted to pH 11 with sodium hydroxide and the basic organic components were extracted with benzene. Two to 3 extractions were carried out as for the acidic compounds.

The water was neutralized with hydrochloric acid and any amphoteric compounds were extracted two to three times with benzene. No detectable change in the chemical composition of the organic constituents resulted from this separation procedure.

Acidic, basic, and neutral organic fractions, separated from water were analyzed by combined gas chromatography-mass spectrometry (GC-MS). Identification of compounds was based on computer comparisons of mass spectra with those of the United States Environmental Protection Agency library, gas chromatographic retention times as compared with standard compounds, and published mass spectra and gas chromatographic data.

Class separation of organic compounds

Produced waters from the in-situ process which were known to be highly toxic by the Microtox test were fractionated and the toxicity of each fraction measured. Volatile components were separated from the sample before fractionation and their contribution to the total toxicity determined.

XAD resins were used to separate the sample into acidic, basic, phenolic and neutral components. These resins are synthetic polystyrene polymers that adsorb nonionized species but do not retain ionized compounds in aqueous solutions. Organic acids are eluted from these resins if the pH is greater than 6; basic components are eluted if the pH is acidic; and phenolic components are eluted if the pH is above 11. Neutral components which do not ionize can be eluted with methanol. Microtox assays can be carried out directly on the acidic, basic and phenolic fractions. The methanol must be removed completely from the neutral fraction and the sample dissolved in water prior to toxicity testing.

The resin bed was prepared by pouring a mixture of 5 g XAD-2 and 5 g XAD-8 into a 25 cm x 1 cm column. The resin was cleaned before use by washing with 100 mL 0.1 N hydrochloric acid, 100 mL pH-7 buffer, 100 mL 0.1N sodium hydroxide and 100 mL methanol. All traces of methanol were removed from the resin bed by extensive washing with water.

Volatile components were removed from the sample by purging with nitrogen for 15 minutes. Fifty mL of the volatile free sample was acidified to 0.1N with 0.5 mL of concentrated hydrochloric acid. The sample was passed through the resin column which retained acidic, phenolic and neutral compounds. The effluent which contained the basic compounds was collected. Fifty mL of 0.1N hydrochloric acid was passed through the column to wash off any remaining basic components. Acidic components were eluted with 50 mL pH-7 buffer, monobasic potassium phosphate-sodium hydroxide. Phenolic compounds were eluted with 50 mL of 0.1N sodium hydroxide. The remaining neutral material was removed with 35 mL of methanol.

RESULTS AND DISCUSSION

Samples (a) as received, (b) after purging with helium or nitrogen to remove volatile organic compounds, (c) after chemical fractionation into acidic, basic, phenolic and neutral compounds, and (d) after extraction of organic compounds were tested for toxicity by the Microtox system. The results, given in Table 2, are expressed as EC_{50} values. Toxic compounds affect the metabolic processes of the bacterial cells reducing the normal light output. The designation EC_{50} is an expression of toxicity defined as the effective concentration of toxicant causing a 50% decrease in the light output of the bacteria. The relative contribution of volatile and extractable compounds or each class of compounds to the overall toxicity can be calculated from the EC_{50} values. A good general agreement has been found between the Microtox bioassay and the fish bioassay with such compounds as benzene, phenol, and trinitrotoluene (Bulich et al., 1981; Qureshi et al., 1982). Studies by Lebsack (1981) on fossil-fuel process waters and on some phenolic compounds indicate the correlation between the Microtox and fish bioassay to be as good as the correlation between two fish bioassays. Low EC_{50} values indicate the presence of significant amounts of toxic compounds whereas values over 80 indicate little or no toxic material.

All samples were toxic as received with EC_{50} values, obtained after 5 minutes of contact between the sample and the bacteria, ranging from 0.30 to 11%. Among the most toxic samples were the COFCAW produced waters, GP-26 sampled in March and May, and the fluids produced by steam stimulation from well GP-31. The acidity of the sample GP-26, May, was adjusted to near pH 7 to avoid any pH effect in the Microtox test. All other samples fell well within the functional pH range of the test. The fluids from GP-31 contained large amounts of dispersed clay minerals and bitumen as indicated by a total organic carbon content of 1,850 ppm (Table 3). Waters from well GP-26 contained lesser amounts of total, dissolved and dispersed, organic carbon but a greater portion of the carbon occurred as volatile organic compounds, 28% in the May sample from well GP-26, as compared with 13% of the carbon in the February sample from well GP-31. In both cases the waters were found to be highly toxic to aquatic organisms. The 5-minute EC_{50} values for the GP-31 samples were 0.30 and 0.31% for the February and March samples, and the values for the GP-26 samples taken in March and May were 0.84 and 0.61%. All the waters produced by both in-situ combustion and steam stimulation were toxic in the Microtox test including shallow groundwaters prior to injection. EC_{50} values measured after 15 minutes did not vary greatly from those measured after 5 minutes.

Purging with helium to remove volatile organic compounds reduced but did not remove the toxicity of the produced waters. Among the major groups of compounds removed from the samples by purging were benzene and alkyl substituted benzenes (Table 4). The toxicity of these; compounds to aquatic organisms is well known; therefore their removal from the samples was expected to reduce toxicity. The samples after purging were still toxic with 5 minute EC_{50} values ranging from 0.39 to 57%. Samples from wells GP-26 and GP-31 were still highly toxic. The water collected from well GP-31 in February contained 1,610 ppm organic carbon after purging and gave a 5-minute EC_{50} value of 0.39%. Removing the purgable organic compounds from sample GP-26 (May) reduced its toxicity, as indicated by an increase in the EC_{50} value from 0.84 to 2.0%. After purging, the raw water showed little toxicity with a value of 76%.

Some toxicity, presumably due to dissolved organic compounds, remained. These organic compounds were then extracted from the produced waters with toluene, a total of nine extractions at three different pH conditions for each sample. After extraction of the organics the aqueous solution was adjusted to its initial pH; thus differences in Microtox EC_{50} values before and after extraction represented the contribution of the organic constituents to the toxicity of the sample. All samples showed a major reduction in toxicity upon extraction and five of the 13 samples produced no significant toxic effect in the Microtox test after extraction. Thus the extractable and volatile organic compounds in the produced waters, rather than the inorganic constituents, were responsible for the majority of the toxicity in the produced waters.

A major group of organic compounds extracted from the waters were phenols: phenol, alkyl substituted phenols, and alkyl substituted naphthalenol (Table 5). These compounds are highly toxic to aquatic organisms. Also found were many alkyl substituted benzoic acids, substituted aromatic hydrocarbons, benzenamines, pyridines and quinolines. The presence of these compounds would readily account for the observed toxicity of the produced waters.

Despite repeated solvent extractions under acid, basic, and neutral conditions, the odor of phenols remained in some of the samples. Apparently not all the phenols were totally extracted from the sample. Consequently three samples were further extracted under acid conditions. These doubly extracted samples were then tested for toxicity. In all cases they produced no significant effect in the Microtox test, confirming that a major cause of toxicity in the produced waters was the extractable organic compounds.

Based upon the average EC_{50} values obtained after 5 and 15 minutes exposure, the contribution of the volatile and extractable compounds to the toxicity were calculated (Table 6). The relative toxicity due to the volatile and extractable compounds varied greatly, from as little as 23% due to volatile compounds to as much as 87%. No meaningful difference was found between waters produced by in-situ combustion and by steam injection. The difficulty in obtaining representative samples of the produced fluids without loss of some volatile materials makes the results questionable. Undoubtedly many volatile components which were in solution in the combustion zone were lost from the samples. A more meaningful measure of the toxicity of the produced fluids is provided by assays after purging of the volatile compounds.

The fractions separated by ion exchange chromatography were examined to determine which chemical classes of components were responsible for the observed toxicity. Averaged results calculated from the EC_{50} 5-minute and 15-minute values are shown in Table 7. In three of the four samples examined the neutral compounds were the major cause of the toxicity, whereas in the steam produced sample taken from well GP-19 in May the acids were the major contributors to the toxicity. The neutral fraction contained aliphatic hydrocarbons C_{11} to C_{25} together with many aromatic compounds. These included alkyl substituted benzenes, indenes, naphthalenes, fluorenes and phenanthrenes, a number of which are known to be toxic to aquatic organisms. The occurrence of these compounds would explain the toxicity of the neutral fraction from the water samples. Similarly the toxicity of the phenolic, acidic, and basic fractions could be explained by the presence of toxic alkyl substituted phenols, substituted benzoic acid and benzenamines, pyridines and quinalines.

SUMMARY AND CONCLUSIONS

Waters produced during the in-situ recovery of oil from the Athabasca oil sands were found to be toxic to aquatic organisms by the Microtox bioluminescence test. EC_{50} values at 15°C as low as 0.30% were found in waters produced by steam stimulation. These waters contained clay minerals, and 1,800 ppm dispersed organic carbon. The toxicity of waters produced by the COFCAW in-situ combustion process was also high. A sample which contained 480 ppm organic carbon gave an EC_{50} value of 0.61%. The toxicity of these waters was caused partly by volatile organic compounds and partly by extractable organic compounds. Inorganic compounds did not contribute to the toxicity. No single class of organic compounds consistently was responsible for the observed toxicity. In the COFCAW produced waters the neutral organic material was the main toxicant.

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TABLES

Table 1. Samples Taken from the Gregoire Lake Pilot Study.

Sample	Description
Water Samples	
GP-20 (Feb) GP-20 (Mar)	Produced water; combination of treated water, raw water and stimulated water
GP-21 (Feb) GP-21 (Mar) GP-21 (Jun)	Produced water; combination of stimulated water and COFCAW water
GP-26 (Feb) GP-26 (Mar) GP-26 (May)	Water produced in combustion process
GP-31 (Feb) GP-31 (Mar)	Produced waters from steam stimulation
GP-19 (May) GP-19 (Jun)	Produced waters from steam stimulation
Groundwater	
Raw Water	Groundwater used for injection

Table 2. The Toxicity of Waters from the Gregoire Lake Pilot Project as Determined with the Microtox System. EC_{50} values at 15°C

Sample	As Received EC_{50} (5) (percent)	As Received EC_{50} (15) (percent)	Volatile Free EC_{50} (5) (percent)	Volatile Free EC_{50} (15) (percent)	Extracted EC_{50} (5) (percent)	Extracted EC_{50} (15) (percent)	Double Extracted EC_{50} (5) (percent)	Double Extracted EC_{50} (15) (percent)
GP-20 (Feb)	7.2	11	21	18	NE*	78		
GP-20 (Mar)	11	11	57	49	NE	NE		
GP-21 (Feb)	2.3	3.0	16	14	89	NE		
GP-21 (Mar)	2.6	3.0	12	8.9	37	36		
GP-21 (Jun)	1.2	1.4	4.6	4.5	81	NE		
GP-26 (Feb)	2.5	3.2	4.6	5.1	26	27	NE	NE
GP-26 (Mar)	0.84	0.96	2.0	2.3	22	18		
GP-26 (May)	2.3	2.3	2.9	3.0	17	16	82	82
GP-31 (Feb)	0.30	0.32	0.39	0.58	33	35	96	88
GP-31 (Mar)	0.31	0.41	0.54	1.4	37	36		
GP-19 (May)	2.9	3.1	17	16	61	65		
GP-19 (Jun)	1.4	1.6	12	11	94	NE		
Raw Water	1.9		76		NE			

*NE - No Significant Effect (5) - 5-minute values (15) - 15-minute values

Table 3. Acidity and Carbon Content of Water Samples

Sample	pH	Volatile Organic Carbon (ppm)	Total Organic Carbon (ppm)
GP-20 (Feb)	6.80	21	116
GP-20 (Mar)	7.10	10	74
GP-21 (Feb)	7.60	11	96
GP-21 (Mar)	8.65	21	103
GP-21 (Jun)	8.55	38	202
GP-26 (Feb)	5.60	21	160
GP-26 (Mar)	6.95	33	170
GP-26 (May)	2.70	136	481
GP-31 (Feb)	7.15	242	1850
GP-31 (Mar)	8.15	82	281
GP-19 (May)	6.70	25	266
GP-19 (Jun)	6.55	26	235
Raw Water	8.60	6	87

Table 4. Volatile Compounds in Sample GP-26 (February)

Scan	M.W.	Compound
245		
272		
286	84	2-methyl-1-pentene
307	78	benzene
325		
333		
359	98	methyl cyclohexane
359		
411	128	4-ethyl-2-methyl hexane
419	92	methyl benzene
435	112	1,2-dimethyl cyclohexane
459	128	2,3,4-trimethyl hexane
541	128	2,4-dimethyl heptane
556	112	ethyl cyclohexane
564	126	1,1,3-trimethyl cyclohexane
582		
600		
610	126	1,3,5-trimethyl cyclohexane
626	128	2,3-dimethyl neptane
649	106	ethyl benzene
686	106	1,3-dimethyl benzene
782	126	3,4,4-trimethyl-2-hexene
828	106	1,4-dimethyl benzene
845	106	1,2-dimethyl benzene
864	128	nonane
879	126	1-ethyl-2-methyl cyclohexane
932	124	1-methyl-octahydro pentalene
963	120	(1-methylethyl)benzene
980	126	propyl cyclohexane
1008	142	3-methyl nonane
1037		
1099	120	propyl benzene
1144	120	1-ethyl-3-methyl benzene
1174	120	1,2,3-trimethyl benzene
1222	120	1-ethyl-4-methyl benzene
1247		
1293	120	1,3,5-trimethyl benzene
1317	142	decane
1348	134	(2-methylpropyl)benzene
1361	134	(1-methylpropyl)benzene
1379		
1417	120	1,2,4-trimethyl benzene
1452	140	butyl cyclohexane
1437	118	1-ethenyl-2-methyl benzene
1488		
1517		
1554	138	decahydro naphthalene
1579	134	1-ethyl-3,5-dimethyl benzene
1601	156	isooctane
1615	134	1-methyl-4-propyl benzene
1631		
1669	134	(1-1-dimethylethyl)benzene
1694		
1705	132	2-butenyl benzene
1766	156	undecane
1787		
1809	152	2-methyl-decahydro naphthalene
1841	134	1,2,3,5-tetramethyl benzene
1860	134	1,2,4,5-tetramethyl benzene
1885	170	4-methyl undecane
1918		
1943	132	1-methyl-2-(2-propenyl)benzene
2005	134	2-ethyl-1,4-dimethyl benzene
2044	132	1,2,3,4-tetrahydro naphthalene
2106	148	(1-1-dimethylpropyl)benzene
2120		
2146	128	naphthalene
2172		
2185	148	diethylmethyl benzene
2194		
2215		
2237		
2261	148	2,4-dimethyl-1-(1-methylethyl)benzene
2273	184	2,6-dimethyl undecane
2297	148	(1,1-dimethyl ethyl)methyl benzene
2306		

Table 5. Acidic Compounds in Sample GP-26 (May)

Scan	M.W.	Compound
287	94	phenol
320	116	hexanoic acid
342	108	2-methyl phenol
364	108	4-methyl phenol
391	122	2,5-dimethyl phenol
416	122	2-ethyl phenol
439	122	2,4-dimethyl phenol
454	122	3-ethyl phenol
478	122	methoxymethyl benzene
500		
513	122	4-ethyl phenol
523	136	ethyl benzenemethanol
542	136	2-ethyl-5-methyl phenol
551	136	4-(1-methylethyl) phenol
562	136	3-ethyl-5-methyl phenol
602	136	benzoic acid
614	136	3,4,5-trimethyl phenol
618	136	2,3,5-trimethyl phenol
625	136	hydrooxyphenyl ethanone
637	158	nonanoic acid
643		
660	136	2-(1-methylethyl) phenol
667		
690	134	2,3-dehydro-1H-inden-1-ol
701	136	methoxy ethyl benzene
731		
748		
763		
777	136	4-methyl benzoic acid
789	150	dimethyl benzoic acid
794		
804	148	2-(2-methyl-2-propenyl) phenol
820	150	2,3,6-trimethyl anisole
844		
870	148	methyl ethyl benzaldehyde
879	150	3-methyl benzoic acid methyl ester
899	150	ethyl benzoic acid
906	148	pentamethyl benzene
922		
934	162	4,6-dimethyl-3(2H)benzofuranone
949	150	3,4-dimethyl benzoic acid
959		
970	150	4-formyl benzoic acid
979		
993	164	4-(1-methylethyl) benzoic acid
1003	176	4,7-dimethyl-1,3-isobenzofurandione
1010	170	1,1'-biphenyl-2-ol
1018	164	3-(2-hydroxyphenyl)2-propenoic acid
1036	144	1-naphthalenol
1043	162	4,7-dimethyl-3(2H)benzofuranone
1055		
1075		
1086	190	3,4-dichloro benzoic acid
1112	162	4,5-dimethyl-3(2H)benzofuranone
1122	164	2,4,5-trimethyl benzoic acid
1133	172	decanoic acid
1150		
1195	158	2-methyl naphthalenol
1208	158	3-methyl naphthalenol
1244	158	4-methyl naphthalenol
1271		
1296		
1311	178	
1350		
1367		
1395		
1412	172	5,7-dimethyl naphthol
1422	172	2-naphthalene carboxylic acid
1431		
1441		
1457		
1472		
1522	184	2-(phenylmethyl) phenol
1531		
1567		
1596		

Table 6. Percent Contribution to the Toxicity of Waters from the Gregoire Lake Pilot Project as Determined with the Microtox System

	Volatile Compounds	Extractable Compounds	Other Compounds
GP-20 (Feb)	52	40	8
GP-20 (Mar)	79	--	--
GP-21 (Feb)	83	15	2
GP-21 (Mar)	73	20	7
GP-21 (Jun)	72	27	1
GP-26 (Feb)	41	57	2
GP-26 (Mar)	58	38	4
GP-26 (May)	26	64	10
GP-31 (Feb)	34	66	0
GP-31 (Mar)	57	42	1
GP-19 (May)	82	13	5
GP-19 (Jun)	87	11	2
Raw Water	98	--	--

Table 7. The Relative Toxicity of Chemical Fractions Separated from Produced Waters as Determined by the Microtox Test

	GP-19 (May)	GP-19 (Jun)	GP-21 (Jun)	GP-21 (May)
Acids	46	10	34	17
Bases	10	23	16	6
Phenols	8	16	9	12
Neutrals	36	51	41	65

IMPLICATIONS OF TOXICOLOGICAL STUDIES FOR THE MARINE DISCHARGE OF LIQUID WASTE FROM A MAJOR ENERGY DEVELOPMENT

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POWER, Fergus M. 1982. Implications of toxicological studies for the marine discharge of liquid waste from a major energy development. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The implications for toxicological assessment of liquid wastes from a proposed synthetic petrol plant at Motunui, on the North Island of New Zealand are discussed. These include the use of 'fast-track' legislation which limited the time available to the authority regionally responsible for licensing activities in the aquatic environment (Taranaki Catchment Commission and Regional Water Board) in which to formulate waste component standards, dispersion criteria and mixing zone size, to six months. Problems encountered were a total absence of toxicological information regarding New Zealand marine species; initially imprecise waste description; and unavailability of the cooling tower water treatment chemicals for testing. The results of acute and sublethal bioassays aimed at establishing waste component standards for the synthetic petrol plant are presented for Zn, Cu, Cr,Pb and the biocide Alfloc 324. The implications of energy-related fast-track legislation for toxicological aquatic hazard assessment are discussed in terms of two scenarios: (a) inadequate protection of the aquatic environment due to insufficient lead-time for investigation; and (b) 'overprotection' imposed by the courts due to the absence of toxicological information regarding a critical waste component (for example, a biocide).

Key Words: Marine species, zinc, copper, chromium, lead, Alfloc 324.

POWER, Fergus M. 1982. Portée d'études toxicologiques sur le déversement dans la mer des effluents liquides provenant d'une importante installation énergétique. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Le présent rapport fait état des conséquences de l'évaluation toxicologique des effluents liquides en provenance d'une usine d'essence synthétique prévue à Motunui, dans l'île du Nord de la Nouvelle-Zélande, pour l'approbation d'un tel projet. Étant donné la loi invoquée pour fiare approuver le projet, les autorités responsables de l'émission de permis pour les exploitations ou activités touchant le milieu aquatique, en l'occurrence le Taranaki Catchment Commission and Regional Water Board, n'avaient que six mois pour arrêter les normes relatives à la composition des effluents, à la dispersion et à l'étendue de la zone de dilution. Elles ne possédaient aucun renseignement sur les espèces marines de la Nouvelle Zélande et n'avaient à leur disposition aucune des substances chimiques requises pour le traitement de l'eau du refroidisseur, substances nécessaires aux expérimentations. En outre, la description des effluents qui leur avait d'abord été remise manquait de précision. Le rapport fait état des résultats des essais biologiques de toxicité aiguë et de sublétalité menés en vue d'établir les normes de composition des effluents provenant de l'usine d'essence synthétique; ces essais portaient sur la teneur en zinc (Zn), en cuivre (Cu), en chrome (Cr), en plomb (Pb) et en biocide Alfloc 324. On discute également les répercussions, sur l'évaluation des risques toxicologiques pour le milieu aquatique, d'une loi visant à accélérer l'approbation de nouveaux projets énergétiques. L'existence de telles conditions peut entraîner deux réactions : a) la protection insuffisante du milieu aquatique par suite d'un manque de temps pour exécuter toutes les études nécessaires ou b) la surprotection de ce milieu, mesure décrétée par les tribunaux en l'absence de données toxicologiques suffisantes pour apprécier correctement une composante essentielle des effluents de l'entreprise (un biocide, par exemple).

INTRODUCTION

The New Zealand Synthetic Fuels Corporation Limited applied for consents to construct a synthetic petrol plant at Motunui, on the western coast of the North Island of New Zealand, in February 1981. These consents were sought under 'fast-track' legislation (the National Development Act 1979) and included the right to discharge a complex liquid industrial waste into the Tasman Sea via a submarine outfall at a rate of 38.6 litres/second. Use of such legislation meant that only six months were available to the authority regionally responsible for licensing activities in the aquatic environment (the Taranaki Catchment Commission and Regional Water Board), in which to formulate waste component standards, dispersion criteria and mixing zone size.

The heart of the synthetic petrol plant complex consists of:

1. two methanol feedstock units, with a combined daily output of 4400 tonnes;
2. the methanol to gasoline conversion plant located adjacent to the methanol plant, and capable of converting methanol to gasoline at a rate of 570 000 tonnes/year.

The outfall component stream flow rates comprise:

(a) cooling towers (30.5 litres/second);
(b) demineralisers, condensate polishers (5.1 litres/second);
(c) sewage (0.6 litres/second); and
(d) raw water treatment sludge lagoons (2.4 litres/second).

A high zinc level (4 mg/L) was expected in the waste stream due to the proposed use of a zinc-based corrosion inhibitor (Nalco 7350) in the cooling towers. Nalco 7350 contains about 10% zinc as zinc chloride plus poly-ol ester.

Effluent component levels are summarised in Table 1. The restricted time available for the investigations meant that each waste component effectively competed with the others for toxicological assessment. A 'hierarchy of competing risks' was established, based upon:

(a) published literature concerning the toxicity of isolated waste components to overseas species;
(b) potential synergism between binary mixtures of major waste components.

The details regarding the selection of the waste components for study is set out in the report of which this paper is a summary (Taranaki Catchment Commission 1981). Toxicological information presented to the Tribunal constituted to consider the water right application to discharge waste from the synthetic petrol plant included:

(a) a review of international toxicological literature dealing with all of the known waste components (except the water treatment chemicals);
(b) results of acute and sublethal tests aimed at assessing the toxicity of zinc, chromium, copper, lead, zinc/copper and zinc/chromium binary metal combinations to a selection of local marine species.

MATERIALS AND METHODS

ADULT STUDIES

Acute bioassays

Selection of test species was based upon five criteria:

(i) importance of the species in the ecology of the shore, particularly in terms of abundance, biomass or dominance;
(ii) consistent presence on the shore throughout the year, thus providing for accessibility and ease of collection;
(iii) the existence of sufficient literature on the ecology of the animal to allow appraisal of its feeding and other behavioural patterns;
(iv) the exclusion of any species which was approaching the boundary of its geographic range;
(v) the ability of the species to survive well under laboratory conditions.

Four species of marine intertidal invertebrate were studied: the carnivorous 'oyster borer' Lepsiella scobina (Quoy and Gaimard 1833); the grazing limpet Cellana radians (Gmelin 1791), and the carnivorous, scavenging and deposit-feeding half-crab Petrolisthes elongatus (Milne Edwards 1837).

All animals were acclimated for 24 hours in oxygenated natural seawater. Animals were not fed. Standard practice of the ASTM (1980) was followed. During each test each species was exposed to a minimum of seven test concentrations and a control. Test solutions were aerated. Test solutions were made immediately before use using AnalaR grade reagents (BDH Chemical Company Limited, Poole, England).

In 48 hr-LC50 tests the solutions were replaced with fresh solution after 24 hours. In 336 hr-LC50 tests, solutions were replaced every alternative day. Approximately fifty (50) animals were used for each test concentration. Animals were placed in PVC aquaria holding 10 litres of test solution at 10 cm depth. Experiments were conducted at various temperatures to assess differences in susceptibility at different temperatures. Animals that died during the exposure period were removed. Following exposure, animals not responding to touch or failing to show respiratory movements were classed as dead. LC50's and LT50's were computed using custom-made programs run on a DIGITAL PDP 11/23 computer. Student's t-test was used to compare the significance of differences between computed LC50 values.

Litchfield and Wilcoxon's (1949) test for parallelism of slopes was used to determine

Table 1 EXPECTED CONCENTRATIONS OF KNOWN EFFLUENT COMPONENTS IN SYNTHETIC PETROL PLANT EFFLUENT

Component	Concentration	
	Initial (ppm)	After 150:1 initial dilution at outfall (ppb)
Natural dissolved salts (from river water)	500	3330
BOD_5	100	667
Phosphates	40	267
Hydrocarbons	≤ 2.7	≤ 18
Alfloc 324 (Biocide)[1]	35[2]	233
Nalco 7348 (Biodispersant)[3]	3,5[2]	23
Nalco 7319 (Dispersant)[4]	8[2]	53
Nalco 7350 (Corrosion inhibitor)[4]	40[2]	266
Chlorine[5]	≤ 0.1	≤ 0.7
Na_2SO_4 from demineralisers/ polishers	533	3553
$(NH_4)_2SO_4$ from demineralisers/ polishers	64	424
Total suspended solids	50	333
pH	6.5-7.5	-
Temperature at outfall sump	20-30°C	-
Trace metals:[6]		
Zn	4.0	27-28
Fe	2.6	19-42
Ni	0.3	3-12
Cr	0.3	2.1-2.2
Cu	0.07	0.64-0.8
Pb	0.03	0.35-0.71
Sn	0.05	0.43
Cd	0.01	0.08-0.12
Mo	0.02	10.1 -12.1

Notes:

[1] Slug - fed once per week to establish a concentration of 50 ppm in recirculating cooling water

[2] Progressive dilution in blowdown holding pond assuming no breakdown of the product

[3] Slug - fed 30 minutes prior to each biocide application

[4] Continual dosage through day to establish a 10 ppm concentration in the recirculating cooling water and blowdown

[5] Applied once per day to establish a 1.0 ppm free residual in the tower return water for 30 minutes

[6] Formula used: seawater concentration

$$= (\text{diffuser dilution factor} \times \text{ocean background level}) + \frac{(\text{effluent dilution} \times \text{effluent concentration of the toxicant})}{(\text{diffuser dilution factor} + \text{effluent dilution factor})}$$

significant differences in probit line slopes. The metals examined in these tests were zinc (as $ZnCl_2$ or $ZnSO_4.7H_2O$), copper (as $CuSO_4.5H_2O$), chromium (as $K_2Cr_2O_7$) and lead (as $(CH_3COO)_2$ $Pb.3H_2O$). In the lead LC50 experiments, LC50's were computed:

(a) immediately after 336 hr-exposure; and
(b) again after a seven-day recovery period.

The latter LC50 figure accounted for consequential deaths following exposure and is considered to be a more realistic LC50.

Marking's Additive Index (MAI) (Marking 1976) was used to establish antagonistic, additive or synergistic effects of binary metal mixtures. Zinc/copper and zinc/chromium mixtures were examined. For each MAI, the three required LC50 tests (metal A, metal B, metal mixture AB) were conducted at similar temperatures. The concentrations of metals used in binary mixtures were in a 1:1 ratio. A quoted binary concentration of 10 mg/L = 5 mg/L metal A and 5 mg/L metal B.

Sublethal studies

The New Zealand edible green-lipped mussel Perna canaliculus (Gmelin 1791) was subjected to a 336 hr-LC50. The results of this and of 1, 5, 10, 50 and 100 mg/L Zn^{2+} (as $ZnSO_4.7H_2O$) LT50's were used to select levels of zinc for use in sublethal behavioural studies. In one experiment, mussels were exposed to either 5 or 50 mg/L Zn^{2+} for 168 hours. In a second, mussels were exposed to 1.0 mg/L Zn^{2+} for 504 hours. Test solutions were replaced every second day. Twenty (20) and forty (40) mussels were used in the control and each treatment for the 5 and 50 mg/L Zn^{2+}, and the 1 mg/L Zn^{2+} experiments respectively. In another experiment, mussels were exposed to eight different concentrations of the biocide Alfloc 324. Concentrations used were: control; 0.1; 0.3; 0.6; 1.0; 2.0; 3.0; 10.0; and 30.0 ml/m^3 Alfloc 324 (Catoleum Pty Limited). These experimental solutions were replaced daily. Three replicate tanks, each holding ten (10) mussels were used for the test solutions, and four replicate tanks each holding ten (10) mussels were used for the control. Mussels were exposed to the Alfloc 324 solutions for four weeks.

In each of these tests, the byssal threads were cut prior to exposure, and at the end of the experiments their growth was measured (number of threads produced and mean length). Clumping behaviour of the mussels was observed. Hourly observations of gaping behaviour were made. Byssal thread attachment to substrate or other mussels was checked and the mussels disattached daily by carefully severing the threads at the point of attachment. At the completion of the zinc experiments, each surviving mussel was opened by severing the adductor muscle. The foot was then prodded, and its reaction observed.

LARVAL STUDIES

Acute bioassays

The method used to test the toxicity of Alfloc 324 to barnacle nauplii makes use of their strong positive reaction to light, and is similar to the method used by Donahue et al. (1977). The nauplius is the first free swimming stage after release from the adults.

Stage I or Stage II nauplii were collected by transporting boulders colonised by adult Elminius modestus to the laboratory. The boulders were submersed in natural unfiltered seawater with a strong light beam passing diagonally through it from above. The nauplii collected at the side of the aquaria at the point where the light was incident. These were collected by pasteur pipette and placed in a small beaker with a darkened bottom and lower sides. Light was applied from directly above and the uniformly dispersed and highly concentrated nauplii were then drawn off for use in the experiments. Only those nauplii which continued to react positively to light were used.

Tests were performed by partially filling a 5 ml syringe with the Alfloc 324 solution at a concentration to be applied to the nauplii. Alfloc 324 was then added to a known volume of seawater (containing the nauplii) in a beaker by micropipette. The nauplii contained in 1.5 ml of this solution were then drawn up into a pasteur pipette, which was subsequently attached to the syringe by a plastic tube. The lower 4 cm of each pasteur pipette was darkened. The pipettes were inclined at an angle of 20^o to the horizontal, and subjected to directional light from above.

At set intervals (normally one, two and three hours), the pipettes were returned to the vertical, tapped vigorously to dislodge any dead animals on the side, and the syringe plunger depressed 0.2 units to expel the bottom fraction. This fraction contained the dead and/or immobilised animals. Use of the attached syringe in this manner meant that the expelled fluid was simultaneously replaced with fresh test solution, preventing a drop in the fluid level in the pasteur pipette and the consequent stranding of nauplii on the glass sides. Tests utilising two replicates were made using: control; 1; 3; 10; 30; 100; 300 and 1000 ml/m^3 Alfloc 324. The numbers of nauplii used in each respective test were: 620; 354; 260; 431; 501; 418; 408 and 199.

The nauplii in the expressed bottom samples were then counted using a binocular microscope. At the conclusion of the sampling periods the numbers of nauplii remaining in the top fraction (healthy nauplii) were counted to allow determination of the initial number of test nauplii.

A comparative test utilising this method was run on Zn^{2+} as $ZnSO_4.7H_2O$.

Table 2 SUMMARISED LC50 TEST CONDITION INFORMATION

Each test number corresponds with that in Table 3

Test No.	Toxin	Species	Mean size ± 95% CI (mm)	Mean temperature (^oC)	Exposure time (hours)	Alkalinity (g/m^3 $CaCO_3$)	Salinity (ppt)	pH
1	$ZnCl_2$	*Cellana radians*	25.67±3.49[1]	17.63±0.4	48	113-123	31	8.1
2	$ZnCl_2$	*Lepsiella scobina*	14.47±2.43[1]	17.63±0.4	48	113-123	31	8.1
3	$ZnCl_2$	*Melagraphia aethiops*	11.51±0.37[2]	17.63±0.4	48	113-123	31	8.1
4	$ZnSO_4.7H_2O$	*C. radians*	22.2 ±2.91[1]	17.15±0.4	48	107	32	8.1
5	$ZnSO_4.7H_2O$	*L. scobina*	12.09±0.47[1]	17.15±0.4	48	107	32	8.1
6	$ZnSO_4.7H_2O$	*Petrolisthes elongatus*	10.19±0.35[3]	17.15±0.4	48	107	32	8.1
7	$ZnSO_4.7H_2O/K_2Cr_2O_7$	*L. scobina*	13.26±0.30[1]	16.86±0.4	48	109	30	8.1
8	$ZnSO_4.7H_2O$	*C. radians*	24.0 ±0.39[1]	13.88±0.4	48	95-109	31	8.1
9	$ZnSO_4.7H_2O/K_2Cr_2O_7$	*C. radians*	24.88±0.87[1]	13.88±0.4	48	95-109	31	8.1
10	$CuSO_4.5H_2O$	*P. elongatus*	9.97±0.32[3]	14.64±0.4	48	109	32	8.2
11	$ZnSO_4.7H_2O$	*L. scobina*	11.45±0.42[1]	13.25±0.4	48	117	31	8.1
12	$ZnSO_4.7H_2O/K_2Cr_2O_7$	*L. scobina*	11.45±0.42[1]	13.25±0.4	48	117	31	8.1
13	$ZnSO_4.7H_2O/K_2Cr_2O_7$	*C. radians*	24.46±1.16[1]	16.67±0.4	48	116	33	8.2
14	$K_2Cr_2O_7$	*C. radians*	24.46±1.16[1]	16.67±0.4	48	116	33	8.1
15	$ZnSO_4.7H_2O/K_2Cr_2O_7$	*L. scobina*	6.57±0.29[2]	16.67±0.4	48	116	33	8.1
16	$K_2Cr_2O_7$	*L. scobina*	6.57±0.29[2]	16.67±0.4	48	116	33	8.1
17	$ZnSO_4$	*C. radians*	25.34±2.08	18.8 ±0.4	336	105-118	32-37	8.0-8.2
18	$ZnSO_4$	*L. scobina*	7.31±0.3[2]	18.8 ±0.4	336	105-118	32-37	8.0-8.2
19	$ZnSO_4$	*P. elongatus*	9.9 ±0.7[3]	18.8 ±0.4	336	105-118	32-37	8.0-8.2
20	$ZnSO_4$	*M. aethiops*	17.7 ±0.3[2]	18.8 ±0.4	336	105-118	32-37	8.0-8.2
21	$(CH_3COO)_2Pb.3H_2O$	*L. scobina*	8.6 ± 0.2[2]	17.0 ±0.4	336	107-125	27-35	8.1
22	$(CH_3COO)_2Pb.3H_2O$	*L. scobina*	8.6 ± 0.2[2]	17.0 ±0.4	336	107-125	27-35	8.1
23	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ± 0.3	17.0 ±0.4	336	107-125	27-35	8.1
24	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ± 0.3	17.0 ±0.4	336	107-125	27-35	8.1

Notes:

[1] Length

[2] Width

[3] Carapace width

Table 3 SUMMARY OF 48 AND 336 HR-LC50 RESULTS

Regression equation: $y = A + B\log_{10}x$

Test No.	Toxin	LC50 (95% CI) (mg/L)		Intercept	Slope	Variance of the slope	Probability
		48 hr:					
1	Zn^{2+} as $ZnCl_2$	5.34	(3.6-8.0)	4.25	1.0254	1.93E-02	0.464E-14
2	Zn^{2+} as $ZnCl_2$	62	(49-77)	1.23	2.11	5.041E-02	0.479E-01
3	Zn^{2+} as $ZnCl_2$	232	(130-420)	2.69	0.98	6.48E-02	
4	Zn^{2+} as $ZnSO_4.7H_2O$	19.4	(13-30)	3.58	1.10	2.52E-02	0.921
5	Zn^{2+} as $ZnSO_4.7H_2O$	54	(45-64)	0.58	2.55	1.20E-01	0.55E-07
6	Zn^{2+} as $ZnSO_4.7H_2O$	23	(9.6-56)	4.16	0.62	4.03E-02	0.52
7	Zn^{2+}/Cr^{6+} as $ZnSO_4.7H_2O/K_2Cr_2O_7$	1204	(653-2219)	1.72	1.06	1.37E-02	0.16E-12
8	Zn^{2+} as $ZnSO_4.7H_2O$	31.8	(23-44)	3.29	1.14	1.05E-02	0.78E-02
9	Zn^{2+}/Cr^{6+} as $ZnSO_4.7H_2O/K_2Cr_2O_7$	42.8	(25.2-72.5)	2.99	1.24	1.17E-02	0.12E-03
10	Cu^{2+} as $CuSO_4.5H_2O$	25.9	(2.6-259)	4.52	0.34	2.86E-02	0.96
11	Zn^{2+} as $ZnSO_4.7H_2O$	375	(230-610)	2.59	0.94	1.57E-02	0.17
12	Zn^{2+}/Cr^{6+} as $ZnSO_4.7H_2O/K_2Cr_2O_7$	309	(176-543)	3.76	0.56	9.59E-03	0.93E-02
13	Zn^{2+}/Cr^{6+} as $ZnSO_4.7H_2O/K_2Cr_2O_7$	11.7	(8.9-15.3)	3.48	1.43	1.42E-02	0.19E-17
14	Cr^{6+} as $K_2Cr_2O_7$	34.04	(27-43)	2.44	1.67	1.73E-02	0.63E-13
15	Zn^{2+}/Cr^{6+} as $ZnSO_4.7H_2O/K_2Cr_2O_7$	133.5	(100.3-177.8)	1.10	1.84	2.76E-03	0.63E-02
16	Cr^{6+} as $K_2Cr_2O_7$	154	(126-187)	-3.79	4.02	1.67E-01	0.83
		336 hr:					
17	Zn^{2+} as $ZnSO_4$	1.09	(0.44-2.7)	4.96	0.96	2.12E-02	0.30E-04
18	Zn^{2+} as $ZnSO_4$	2.94	(2.48-3.48)	2.00	6.40	5.87E-01	0.21E-07
19	Zn^{2+} as $ZnSO_4$	0.25	(0.2-0.3)	6.45	2.43	4.28E-02	0.42E-03
20	Zn^{2+} as $ZnSO_4$	0.45	(estimated graphically)				
21	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$	111	(67-185)	0.89	2.00	9.71E-02	0.20
22	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (after seven day recovery)	29	(17-50)	3.94	0.72	6.18E-03	0.18E-10
23	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$	1.4	(1.3-1.6)	3.78	8.05	7.75E-01	0.14E-02
24	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (after seven day recovery)	0.85	(0.7-1.0)	5.49	7.18	6.96E-01	0.79

RESULTS AND DISCUSSION

ADULT STUDIES

Acute bioassays

The conditions and results of the 48 hr and 336 hr-LC50 tests of Zn, Cu, Cr and Pb toxicity to the four marine intertidal invertebrates under study are given in Tables 2 and 3.

Zinc toxicity: All of the test species demonstrated increased susceptibility to zinc with increasing temperature. In 48 hr-LC50 tests, the slope functions for each Cellana radians probit were quite similar, despite the different compounds that the zinc was applied as and the different test temperatures employed. $ZnCl_2$ was significantly more toxic to C. radians than $ZnSO_4$ ($P < 0.001$). In 48 hr-LC50 tests involving Lepsiella scobina, $ZnSO_4$ was significantly more toxic than $ZnCl_2$ ($P < 0.001$). At 17.15°C, $ZnSO_4$ was almost seven times more toxic to L. scobina than at 13.25°C. Lepsiella scobina demonstrated greater slope functions than C. radians or Melagraphia aethiops. The slope functions for L. scobina increased with increasing temperature. The most obvious implication of the widely differing slope functions for each respective probit is that the rank order of species susceptibility to zinc changes with increasing concentration.

In 336 hr-LC50 tests, greatly reduced Zn levels were required to produce LC50's for all test species. In 18.8°C tests, both L. scobina and M. aethiops demonstrated large slope functions (that is, a rapid change in mortality over a narrow concentration span). In the case of M. aethiops mortality increased from nil in 0.22 mg/L Zn^{2+} to 95 per cent in 0.62 mg/L Zn^{2+}. Since only one partial mortality occurred (with nil and 100 per cent mortalities below and above these concentrations) an LC50 could not be computed. The 336 hr-LC50 for M. aethiops was derived graphically using a standard method (American Public Health Association 1976).

A comparison of the rank order of each species (judged by LC50's) for 48 hr and 336 hr-LC50's (in order of increasing resistance) is given below (Table 4).

Table 4 COMPARISON OF RANK ORDER OF SPECIES RESISTANCE TO ZINC. SPEARMAN'S RANK-CORRELATION COEFFICIENT: T = 0, CORRELATION = 0

Species	48 hr-LC50	336 hr-LC50
Cellana radians	1	3
Petrolisthes elongatus	2	1
Lepsiella scobina	3	4
Melagraphia aethiops	4	2

LT50 tests were particularly difficult to perform with gastropods and limpets because of the difficulty in determining the state of the animals without unduly interfering with them. However, individual checking of each animal at broadly spaced time intervals still allowed useful analysis. The results presented for P. elongatus are substantially more complete due to the easily-observed nature of their scaphognathite activity.

LT50 test conditions and results are given in Tables 5 and 6. There was a clear trend that with increasing Zn^{2+} concentration, less time was required for the achievement of the LT50. Although a longer time for reaction was required with lower concentrations, once reaction began it was more rapid in lower than in higher concentrations. Comparison of LT50 test concentrations with computed slope functions using Spearman's rank-correlation coefficient produced: $T = -3.58$ and a correlation of -0.9.

Size-specific and sex-specific differential mortality: After the conclusion of several acute zinc toxicity tests, randomly selected animals were measured using an appropriate parameter. Student's t-tests were conducted to find significant differences between surviving and dead animals of each species, and for differences in the sizes of live and dead male and female P. elongatus.

In both 48 hr and 336 hr-LC50 tests, dead C. radians were significantly larger than the survivors. The opposite was the case for L. scobina, while no significant size-differential mortality was detected for M. aethiops. Results varied for P. elongatus, but it should be noted that in 48 hr-LC50 tests, significantly more smaller females died than larger ones did. This phenomenon was not detected by t-test in the results of 336 hr-LC50 experiments.

Chromium toxicity: In 48 hr-LC50 tests, Cr^{6+} proved to be 4.5 times more toxic to C. radians than to L. scobina (Tables 2 and 3).

Copper toxicity: In 48 hr-LC50 tests, Cu^{2+} proved to be more toxic to P. elongatus (Tables 2 and 3) than to L. scobina. The 14.6°C 48hr-LC50 (Cu^{2+} as $CuSO_4.5H_2O$) for L. scobina is >30 mg/L Cu^{2+}, the highest concentration used in the bioassay. At 14.64°C Cu^{2+} as $CuSO_4.5H_2O$ demonstrated a similar toxicity to P. elongatus to that of Zn^{2+} as $ZnSO_4.7H_2O$ at 17.15°C (48 hr-LC50's of 25.9 and 23.0 mg/L respectively) ($P = 0.33$).

Lead toxicity: In 336 hr-LC50 tests, L. scobina resisted lead levels 100 times higher than those tolerated by M. aethiops. A large number of animals died subsequently to exposure, during the seven day recovery period. The occurrence of such extended consequential mortality in effluent-exposed animals after acute exposure is of great importance since it indicates that LC50's obtained in the laboratory without the provision of a recovery period could be overestimated, and consequently that discharge of the effluent in the field may induce heavier mortalities than expected. When LC50 values were computed after taking a seven-day recovery period into consideration, a marked reduction in the LC50 values was obtained (Table 3). These LC50 figures accounted for consequential deaths following exposure and are considered to be more realistic LC50's.

Binary metal mixtures: It is not possible to predict, from studies involving isolated metals, the toxicity of binary or more complex mixtures of metals, nor is it possible to predict, from the known toxicity of a binary mixture to a particular species, the toxicity of the same metal mixture to another species. The same binary mixture may demonstrate antagonistic, additional or synergistic effects depending upon the species used for the test (Taranaki Catchment Commission 1981).

The New Zealand Synthetic Fuels Corporation Limited applied for a water right to discharge a complex industrial waste with nine trace metals as components (Table 1). The most significant of these metals in terms of concentration was zinc. Investigations of the toxicity of binary metal mixtures were conducted using several species. Zinc/copper and zinc/chromium combinations were studied, since chromium and copper were examples of metals, other than zinc, likely to be found at concentrations significantly higher than ambient seawater levels after initial dilution at the submarine outfall.

Zinc/copper mixtures: A 14.6°C 48 hr-LC50 (Zn^{2+} as $CuSO_4$) was conducted on *L. scobina* (alkalinity = 109 gm^{-3} $CaCO_3$, salinity = 34 ppt, pH = 8.2), but only 9 per cent mortality was achieved at the highest concentration used (50 mg/L $Zn^{2+} + Cu^{2+}$). Thus the 14.6°C 48 hr-LC50 (Zn^{2+} as $ZnSO_4/Cu^{2+}$ as $CuSO_4$) for *L. scobina* was >50 mg/L Zn^{2+}/Cu^{2+}.

A similar test was conducted for *P. elongatus*, using identical conditions, and in this instance only 29 per cent mortality was achieved at the highest concentration used (50 mg/L $Zn^{2+} + Cu^{2+}$). Thus the 14.6°C 48 hr-LC50 (Zn^{2+} as $ZnSO_4/Cu^{2+}$ as $CuSO_4$) for *P. elongatus* was >50 mg/L $Zn^{2+} + Cu^{2+}$.

Zinc/chromium mixtures: The 13.88°C 48 hr-LC50 (Zn^{2+} as $ZnSO_4/Cr^{6+}$ as $K_2Cr_2O_7$) for *C. radians* was 42.8 (25.2-72.5) mg/L. Increased temperature significantly reduced the LC50 figure. The 16.67°C 48 hr-LC50 (Zn^{2+} as $ZnSO_4/Cr^{6+}$ as $K_2Cr_2O_7$) was 11.7 (8.9-15.3) mg/L. The result of this test was combined with a 17.15°C $ZnSO_4$ (Test No 4) and a 16.67°C $K_2Cr_2O_7$ test (Test No 14) to calculate a Marking's Additive Index for the binary mixture. The calculated S-value was 0.94, and gave a MAI of 0.06, and a magnification factor of 1.06. The range of the index was 0.94 to 1.49. The mixture was therefore demonstrated to be synergistic.

The 13.25°C, 16.86°C and 16.67°C 48 hr-LC50's (Zn^{2+} as $ZnSO_4/Cr^{6+}$ as $K_2Cr_2O_7$) for *L. scobina* were 309 (176-543), 1204 (653-2219) and 134 (100-178) mg/L respectively. The result of the latter test was combined with a 17.15°C $ZnSO_4$ test and a 16.67°C $K_2Cr_2O_7$ test to calculate a MAI for the binary mixture. The calculated S-value was 3.32, and gave a MAI of -2.32 and a magnification factor of -1.32. The range of the index was 3.32 to 3.49. The mixture was therefore demonstrated to be antagonistic.

At concentrations below 800 mg/L the Zn^{2+}/Cr^{6+} mixture was more toxic to *L. scobina* than Zn^{2+} in isolation. With increasing concentration their relative toxicities converged and then reversed. At lower temperatures the Zn^{2+}/Cr^{2+} mixture appeared to be more toxic than Zn^{2+}, whereas the reverse was true for higher temperatures.

Two aspects of the zinc/chromium binary mixture toxicity experiments are of interest: firstly, there was a change in the relative toxicity of the mixture when compared to that of zinc alone, with changing concentration; and secondly, the synergistic effect of the Zn^{2+}/Cr^{6+} mixture manifested in the *C. radians* results contrasted with the antagonistic effect of the same mixture manifested in the *L. scobina* results. With increasing Zn^{2+}/Cr^{6+} concentrations, the pH of the test medium decreased while alkalinity increased. For example, on the completion of a 13°C 48 hr-LC50 test, the pH and alkalinity of a 500 mg/L ($Zn^{2+} + Cr^{6+}$) test solution were 6.3 and 173 gm^{-3} $CaCO_3$ respectively, as opposed to the control (8.1 and 119 gm^{-3} $CaCO_3$ respectively). It is possible that the reduced pH of higher test concentrations may have augmented the availability of free metal ions.

Sublethal studies: The conditions and results of a 12.35°C 336 hr-LC50 (Zn^{2+} as $ZnSO_4$) for *Perna canaliculus* are given in Tables 7 and 8 (Test No 60). LT50 test results are also provided in these tables. The LT50 test results are particularly interesting since not only do the 5, 10, 50 and 100 mg/L Zn^{2+} LT50's have similarly large slope functions, but when plotted as probits, were virtually indistinguishable. While the 1 mg/L Zn^{2+} 12.35°C LT50 produced very different results, with increased temperature (19.46°C) the probit for 1 mg/L Zn^{2+} closely approached those for 5 to 100 mg/L Zn^{2+} at the lower temperature. These results imply that at a particular temperature, the same LT50 response will be elicited in zinc concentrations ranging from 5 to 100 mg/L Zn^{2+}.

There were marked differences in the behaviour of control and experimental mussels. Control mussels produced new byssal threads and clumped together. None of the experimental mussels shared this gregarious behaviour.

Due to the obvious effect of temperature on *Perna canaliculus* sensitivity to zinc, two low-temperature (13.62°C) sublethal studies were initiated using 5 and 50 mg/L Zn^{2+} as $ZnSO_4$.

Table 5 SUMMARISED LT50 TEST CONDITION INFORMATION

Each test number corresponds with that in Table 6

Test No.	Toxin	Species	Mean size ± 95% CI (mm)	Mean temperature ture (°C)	Alkalinity (g/m^3 CaCO$_3$)	Salinity (ppt)	pH
25	$ZnSO_4$	*Cellana radians*	25.34±2.08	18.76±0.35	105-118	31.6-36.6	8.0-8.2
26	$ZnSO_4$	*C. radians*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
27	$ZnSO_4$	*C. radians*	18.47±0.82	13.78±0.54	111		8.2
28	$ZnSO_4$	*C. radians*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
29	$ZnSO_4$	*Lepsiella scobina*	13.26±0.389	13.78±0.54	111		8.2
30	$ZnSO_4$	*Melagraphia aethiops*	17.18±0.48	18.76±0.35	105-118	31.6-36.6	8.0-8.2
31	$ZnSO_4$	*M. aethiops*	17.11±0.5	18.76±0.35	105-118	31.6-36.6	8.0-8.2
32	$ZnSO_4$	*M. aethiops*	17.65±0.4	18.76±0.35	105-118	31.6-36.6	8.0-8.2
33	$ZnSO_4$	*M. aethiops*	17.75±0.4	18.76±0.35	105-118	31.6-36.6	8.0-8.2
34	$ZnSO_4$	*M. aethiops*	17.64±0.42	18.76±0.35	105-118	31.6-36.6	8.0-8.2
35	$ZnSO_4$	*M. aethiops*	17.99±0.37	18.76±0.35	105-118	31.6-36.6	8.0-8.2
36	$ZnSO_4$	*M. aethiops*	14.16±0.3	13.78±0.54	111		8.2
37	$ZnSO_4$	*M. aethiops*	17.67±0.32	18.76±0.35	105-118	31.6-36.6	8.0-8.2
38	$ZnSO_4$	*M. aethiops*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
39	$ZnSO_4$	*Petrolisthes elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
40	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
41	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
42	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
43	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
44	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
45	$ZnSO_4$	*P. elongatus*	9.15±0.56	13.78±0.54	111		8.2
46	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
47	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2
48	$ZnSO_4$	*P. elongatus*		18.76±0.35	105-118	31.6-36.6	8.0-8.2

Table 5 (continued)

Test No.	Toxin	Species	Mean size ± 95% CI (mm)	Mean temperature ture (^{o}C)	Alkalinity (g/m^3 $CaCO_3$)	Salinity (ppt)	pH
49	$(CH_3COO)_2Pb.3H_2O$	*L. scobina*	8.6 ±0.2	17.0 ±0.24	107-125	27-35	8.1
50	$(CH_3COO)_2Pb.3H_2O$	*L. scobina*	8.6 ±0.2	17.0 ±0.24	107-125	27-35	8.1
51	$(CH_3COO)_2Pb.3H_2O$	*L. scobina*	8.6 ±0.2	17.0 ±0.24	107-125	27-35	8.1
52	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
53	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
54	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
55	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
56	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
57	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
58	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1
59	$(CH_3COO)_2Pb.3H_2O$	*M. aethiops*	18.4 ±0.3	17.0 ±0.24	107-125	27-35	8.1

Table 6 SUMMARY OF LT50 RESULTS

Regression equation: $y = A + B\log_{10}x$

Test No.	Toxin (concentration mg/L)	LT50 (95% CI) (hours)	Intercept	Slope	Variance of the slope	Probability
25	Zn^{2+} as $ZnSO_4$ (0.02)	3052 (618-15 080)	1.64	0.96	0.06	1.00
26	Zn^{2+} as $ZnSO_4$ (0.03)	no mortality after 329 hours				
27	Zn^{2+} as $ZnSO_4$ (0.05)	3488 (187-65 000)	1.10	1.10	0.29	0.97
28	Zn^{2+} as $ZnSO_4$ (1.04)	267 (247-288)	-2.91	3.26	0.59	0.17
29	Zn^{2+} as $ZnSO_4$ (0.60)	no mortality after 336 hours				
30	Zn^{2+} as $ZnSO_4$ (0.02)	no mortality after 329 hours				
31	Zn^{2+} as $ZnSO_4$ (0.05)	no mortality after 329 hours				
32	Zn^{2+} as $ZnSO_4$ (0.22)	no mortality after 329 hours				
33	Zn^{2+} as $ZnSO_4$ (0.62)	253 (248-258)	-38.9	18.28	1.81	0.52
34	Zn^{2+} as $ZnSO_4$ (1.04)	151 (143-159)	-19.29	11.15	1.03	0.64
35	Zn^{2+} as $ZnSO_4$ (1.4)	138 (128-149)	-17.99	10.73	1.58	0.32
36	Zn^{2+} as $ZnSO_4$ (2.3)	129 (6- 2670)	-6.13	5.27	22.15	0.57
37	Zn^{2+} as $ZnSO_4$ (2.30)	99 (75-132)	-1	8.38	4.08	0.77
38	Zn^{2+} as $ZnSO_4$ (5.0)	97 (64-149)	-13.18	9.13	6.64	0.85
39	Zn^{2+} as $ZnSO_4$ (0.02)	no mortality after 329 hours				
40	Zn^{2+} as $ZnSO_4$ (0.03)	6% mortality after 329 hours				
41	Zn^{2+} as $ZnSO_4$ (0.05)	no mortality after 329 hours				
42	Zn^{2+} as $ZnSO_4$ (0.07)	6% mortality after 329 hours				
43	Zn^{2+} as $ZnSO_4$ (0.13)	5092(1038-24 970)	1.84	0.85	0.03	0
44	Zn^{2+} as $ZnSO_4$ (0.22)	598 (407-879)	-2.45	2.68	0.27	1.0
45	Zn^{2+} as $ZnSO_4$ (0.23)	874 (523-1530)	-2.18	2.43	0.22	0.96
46	Zn^{2+} as $ZnSO_4$ (0.37)	207 (195-222)	-2.79	3.36	0.05	0.55
47	Zn^{2+} as $ZnSO_4$ (0.62)	131 (113-131)	-1.78	3.26	0.04	0
48	Zn^{2+} as $ZnSO_4$ (2.3)	29 (20-43)	1.97	2.06	0.06	0

Table 6 (continued)

Test No.	Toxin (concentration mg/L)	LT50 (95% CI) (hours)	Intercept	Slope	Variance of the slope	Probability
49	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (31)	389.46(315.3-481)	-29.56	13.33	13.68	0.74
50	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (56)	423.93(243.6-737.8)	-22.67	16.53	18.01	0.52
51	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (100)	324.17 (301-349.2)	-16.188	8.44	1.17	0.94
52	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (2)	261 (252-270)	-14.48	8.06	0.27	0.0000
53	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (3)	246 (238-256)	-21.78	11.20	0.83	0.02
54	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (5)	267 (257-278)	-19.32	10.02	0.79	0.0000
55	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (10)	206 (195-217)	-9.24	6.16	0.26	0.0000
56	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (17)	165 (155-177)	-6.84	5.34	0.16	0.0000
57	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (31)	157 (148-167)	-11.15	7.36	0.32	0.0000
58	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (56)	195 (184-206)	-8.87	6.06	0.23	0.0000
59	Pb^{2+} as $(CH_3COO)_2Pb.3H_2O$ (100)	142 (135-151)	-16.20	9.84	0.81	0.0000

Table 7 SUMMARISED LC50 AND LT50 TEST CONDITION INFORMATION FOR *PERNA CANALICULUS*

Each test number corresponds with that in Table 8

Test No.	Toxin	Mean size ± 95% CI (mm)	Mean temperature ture (°C)	Exposure time (hours)	Alkalinity (g/m^3 $CaCO_3$)	Salinity (ppt)	pH
60	Zn^{2+} as $ZnSO_4.7H_2O$	55.75+1.45	12.35+0.22	336	102-116	25-32	7.6-8.2
61	Zn^{2+} as $ZnSO_4.7H_2O$	67.81±2.05	19.46±0.40	-	113-119	34-35	8.1-8.2
62-66	Zn^{2+} as $ZnSO_4.7H_2O$	55.75±1.45	12.35±0.22	-	102-116	25-32	7.6-8.2

Table 8 SUMMARY OF LC50 AND LT50 RESULTS FOR *PERNA CANALICULUS*

Regression equation: $y = A + B\log_{10}x$

Test No.	Toxin	336 hr-LC50 (95% CI) (mg/L)	Intercept	Slope	Variance of the slope	Probability
60	Zn^{2+} as $ZnSO_4.7H_2O$	19 (9-42)	3.15	1.44	0.06	0.34

Test No.	Toxin (concentration mg/L)	LT50 (95% CI) (hours)	Intercept	Slope	Variance of the slope	Probability
61	Zn^{2+} as $ZnSO_4.7H_2O$ (1.0)	475 (448-504)	-18.31	8.71	0.96	0.06
62	Zn^{2+} as $ZnSO_4.7H_2O$ (1.0)	15% mortality after 504 hours				
63	Zn^{2+} as $ZnSO_4.7H_2O$ (5.0)	358 (343-374)	-39.08	17.26	6.32	0.23
64	Zn^{2+} as $ZnSO_4.7H_2O$ (10.0)	327 (312-342)	-47.12	20.73	10.67	0.23
65	Zn^{2+} as $ZnSO_4.7H_2O$ (50.0)	330 (316-346)	-40.05	17.88	7.56	0.99
66	Zn^{2+} as $ZnSO_4.7H_2O$ (100.0)	289 (271-308)	-30.21	14.31	4.82	0.57

Table 9 SUMMARISED SUBLETHAL TEST CONDITION INFORMATION FOR *PERNA CANALICULUS* EXPERIMENTS

Test No.	Toxin (concentration mg/L)	Mean size ± 95% CI (mm)	Mean temperature ture (°C)	Alkalinity (g/m^3 CaCO$_3$)	Salinity (ppt)	pH
67	Zn^{2+} as ZnSO$_4$.7H$_2$O (50)	54.05±3.49	13.39±0.57	110-113	25	8.2
68	Zn^{2+} as ZnSO$_4$.7H$_2$O (5)	54.62±3.16	13.79±0.26	110-113	25	8.2
69	Zn^{2+} as ZnSO$_4$.7H$_2$O (1)	67.8 ±2.05	19.46±2.4	113-119	34-35	8.1-8.2

One high temperature ($19.46^{\circ}C$) study was undertaken using 1 mg/L Zn^{2+} as $ZnSO_4$. Test conditions are summarised in Table 9.

Gaping behaviour: Immediately following exposure the 50 mg/L Zn^{2+} mussels showed a marked difference in the percentage of animals gaping (variable, 0 to 20 per cent) when compared with the control (variable, 60 to 100 per cent). This difference continued for the duration of the exposure (168 hours). In contrast, 5 mg/L Zn^{2+}-exposed mussels continued to gape normally for up to four days, then showed a gradual decline in gaping activity (variable, 30 to 80 per cent).

This effect of zinc on filtering behaviour of the mussel highlights one of the problems encountered when conducting toxicity tests with animals such as bivalves, which can deliberately isolate themselves from the environment in response to the presence of a pollutant in high concentrations. While it might be expected that the 50 mg/L Zn^{2+} exposed mussels would show mortality much earlier than the 5 mg/L Zn^{2+}-exposed mussels, this was not the case. Whereas the 50 mg/L Zn^{2+}-exposed animals immediately reacted appropriately to the toxin and reduced time spent filtering to a minimum, thus reducing overall exposure, the 5 mg/L Zn^{2+}-exposed mussels continued to filter normally, and although exposed to a much lower concentration of zinc, probably suffered a similar overall dosage due to an initial lack of the avoidance response (shutting) of the valves, cessation of filtering activity, and exclusion of the external medium). After about 40 hours exposure, the 5 mg/L Zn^{2+}-exposed mussels became progressively slower in initiating their closing response after prodding. Narcosis increased with time. Upon completion of the seven-day exposure period, none of the 5 mg/L Zn^{2+}-exposed mussels and only two of the 50 mg/L Zn^{2+}-exposed mussels, had died.

Byssal thread production was significantly inhibited in 50 mg/L Zn^{2+}-exposed mussels ($P < 0.05$). None of the byssal threads produced by the 5 and 50 mg/L Zn^{2+}-exposed mussels successfully attached to the substrate. The average lengths of the byssal threads produced ($\pm$95% CI) were (mm): control, 11.02 ± 1.83; 5 mg/L Zn^{2+}, 10.33 ± 1.64; 50 mg/L Zn^{2+}, 8.62 ± 1.29.

At the end of these experiments, each mussel was opened by severing the adductor muscle. The foot was then prodded, and its reaction observed. The following scale was used to denote reaction of the foot to stimulation:

0: no response

1: slow response

2: rapid response

The results are shown in Table 10.

Table 10 RESPONSE OF THE FOOT MUSCLE OF PERNA CANALICULUS AFTER A SEVEN-DAY EXPOSURE TO ZINC

	Type of response		
	0	1	2
Control	0	1	19
5 mg/L Zn^{2+}	0	14	6
50 mg/L Zn^{2+}	3	17	0

Such narcosis is of obvious disadvantage in the case of predatory attack. Mussels showing no response at all were demonstrated to be alive by checking for filtering activity. This was done by dropping powerdered graphite before the inhalant and exhalant siphons, and observing the respiratory currents.

These sublethal responses were examined at a lower zinc concentration (1 mg/L Zn^{2+} as $ZnSO_4.7H_2O$) and higher temperature ($19.46 \pm 0.24^{\circ}C$) Forty (40) control and forty (40) experimental mussels were observed for 504 hours. There was no significant difference in filtering behaviour between control and experimental mussels over the duration of the experiment. A greater number of control mussels rejected their byssal threads in the first few days than the experimental mussels. All of the control mussels that rejected their threads grew new threads, while only 25 per cent of the experimental mussels that rejected their byssal threads showed signs of replacement. Comparison of byssal thread growth and attachment and foot response is provided in Table 11.

These results demonstrate that while the majority of mussels may survive such zinc concentrations in the laboratory, their behaviour is modified in several ways which would be likely to cause mortality in the field (failure to secrete byssal threads; failure to attach to the substrate; inducement of abnormal filtering behaviour; and general narcosis causing failure to respond to touch).

Effluent standards for the synthetic petrol plant: In August 1981 the Planning Tribunal constituted to hear evidence pertaining to the various consents sought by the New Zealand Synthetic Fuels Corporation Limited under fast-track legislation (the National Development Act 1979) received evidence regarding the potential environmental impact of waste discharged via the submarine outfall to be constructed in association with the plant.

The Taranaki Catchment Commission and Regional Water Board, having regard for the fact that both C. radians and P. elongatus exhibited mortality in 20 µg/L Zn^{2+} as $ZnSO_4.7H_2O$ during the 336 hr-LC50 tests, and noting that there was no reason to assume that an asymptote in toxicity had been reached at this concentration (the lowest tested), considered that such a level of

Table 11 BYSSAL THREAD GROWTH AND ATTACHMENT IN *PERNA CANALICULUS*. CONTROL VERSUS 1 MG/L Zn^{2+}-EXPOSED MUSSELS

	Control	1 mg/L Zn^{2+}
Average length of byssal threads ($\pm$95% CI) (mm)	20.16$\pm$2.4	9.91$\pm$0.93
Number of mussels	32	23
Number of threads	328	898
Number of threads/mussel	10.25	39
Total number of byssal threads attached to substrate	1127 (n =40)	18 (n = 7)
Average number of successful byssal thread attachments/ mussel	28	3
Foot response: 0	0	21 (all dead)
1	2	11
2	38	8

zinc represented the maximum allowable level in the receiving waters. However, in view of the known greater sensitivity of larvae to trace metal toxicity, it was considered appropriate to define a mixing zone of 300 metres radius from the centre point of the outfall diffuser, beyond which an even stricter condition would apply with regard to elevation of zinc levels in the receiving water.

Standards for effluent components were set both for the waste stream and for the receiving waters. The standards set for the waste stream were essentially the maximum levels provided by the Applicant in the Environmental Impact Report for the plant. The water right condition governing the waste stream components is set out below:

Special condition 13
That on the basis of 24-hour flow-proportioned composite samples components of the effluent stream as specified by the Grantee shall not exceed the following total concentrations:

Component	Maximum (g/m^3)
Iron	3.00
Zinc	4.00
Chromium	0.30
Cadmium	0.02
Lead	0.10
Nickel	0.50
Copper	0.30
Phenols	0.01
Free chlorine residual	0.20
Halogenated hydrocarbons	0.01
Methanol	10.00
Molybdenum	0.02
Tin	0.05
Hydrocarbons (less than)	5.00
Alfloc 324 biocide	35.00
Nalco 7348 biodispersant	3.50
Nalco 7319 dispersant	8.00
Nalco 7350 corrosion inhibitor	40.00

The outfall was required to have a minimum initial dilution of 1:150.

The condition regulating the effect of the discharge upon the receiving waters incorporates several standards. These were derived by consideration of:

(a) toxicological studies conducted by the Taranaki Catchment Commission and Regional Water Board involving local species; and

(b) a review of international toxicological literature (Taranaki Catchment Commission 1981).

The mixing zone and receiving water condition are set out below:

Special condition 22
That there shall be no contamination of marine waters such that local populations of aquatic life are significantly damaged or rendered unfit for human consumption by reason of the effect of this discharge beyond the edge of a zone of 300 metres radius from the centre point of the diffuser;

Special condition 23
That the discharge of waste by the Grantee shall not cause the following receiving water quality limits to be exceeded at any place outside the 300 metre mixing zone as detailed in (22) above:

Component	Maximum (mg/m^3)
Zinc	10
Nickel	2
Chromium	1.0
Copper	0.5
Lead	0.5
Tin	0.2
Cadmium	0.03
Molybdenum	15
Un-ionized ammonia	25
pH	No variation from ambient pH by 0.2 units
Dissolved sulphides	100

These and several other recommendations regarding conditions pertaining to the discharge were adopted without change by the Planning Tribunal when it announced its consents for the project.

Environmental controversy and 'fast-track' legislation: Having completed an eight week enquiry into the application to construct the synthetic petrol plant, the Planning Tribunal prepared a report and forwarded this, along with associated recommendations on various consents to the Minister for National Development. With regard to disposal of the liquid waste from the plant via a marine outfall, the Planning Tribunal reported that

> 'Apart from the total concept of the plant, which is unacceptable to many objectors, this aspect of the proposal gave rise to major controversy....The general Motunui reef system contains an abundance of sea life which is an important food source for both the Maori and the European races. The Te Atiawa Tribe and its hapus have historic associations with the coastline in this area and depend upon the sea resources to provide them with the diet to which they have been accustomed for many centuries. Each hapu has its own particular reef or area and tribal custom discourages members of one hapu from gathering food from the reef of another hapu. Thus the contamination of one reef would deprive the hapu which customarily was entitled to the sea food from that reef.

> Although the law does not prevent the gathering of sea food from anywhere along the coast, the evidence indicated that Maori custom, which is very strong amongst the members of the Te Atiawa Tribe, would act as an effective social prohibition.
>
> The Maori people treat the reefs with the greatest of respect insofar as cleanliness is concerned: there are stringent tribal rules concerning the personal hygiene of the sea food gatherers which are incompatible with any discharge of sewage effluent into the ocean, no matter how well such effluent is treated.'
>
> '....The Tribunal receives evidence for the purpose of making a decision. The total impression left with us is that investigation into the effects of zinc has not proceeded to a stage where any expert can with absolute confidence state that sublethal damage would not occur within this important marine environment. We have further concluded that the potential effects of the biocide Nalco 7330 [equivalent to Alfloc 324] on marine life are largely unknown. When we add these two factors to the possibility of accidental spillage of a compound such as Nalco 7330 into the discharge system, we are far from convinced that the suggested discharge point is far enough away from the reef system to guarantee protection of marine life. We have no evidence on the engineering effects of any additional length of pipeline or whether such an addition is feasible. We have, however, concluded that we cannot recommend the discharge as at present suggested in the absence of further research.' (Planning Tribunal 1981)

The Taranaki Catchment Commission and Regional Water Board was unable to toxicologically assess the biocide prior to the Planning Tribunal hearing due to the inability of the New Zealand Synthetic Fuels Corporation Limited to procure a sample. The public controversy surrounding the biocide was based on a fear of extreme toxicity to marine life, particularly edible seafoods; and a generally-held assumption that zinc and the biocide would interact synergistically.

In the absence of information to the contrary, the Planning Tribunal assumed a cautious attitude, recommending a more remote discharge location. The outfall was extended in length by 300 metres, a decision likely to cost the Applicant in excess of NZ $1.5 million.

Working under the constraints imposed by 'fast-track' legislation both statutory authorities and Planning Tribunals must collect, synthesise and digest a wide range of information dealing with often complex projects. Decisions regarding consents, especially those involving the discharge of complex wastes, may be made using an inadequate data base. In the absence of sufficient toxicological information, a cautious approach to effluent dilution factors and receiving water effluent component standards is wise. However, mere absence of information regarding an effluent component does not imply that it represents an extreme hazard. In such situations the decision-making process should follow the pattern described in Figure 1.

The 'fast-track' legacy: Acceptability of a discharge rests on two conditions:

1. dilution of the substances which occur naturally in the ocean to near-ambient levels at which no sublethal toxicity will be experienced by marine organisms;
2. dilution and dispersion of potentially accumulable waste components which may pose a threat to:
   (a) marine organisms;
   (b) human consumers of contaminated sea foods.

Acting on the unproven assumption that the biocide to be used in the synthetic petrol plant was highly toxic to marine organisms, the Planning Tribunal fulfilled the above conditions by recommending an extension in the length of the outfall. The lack of such caution when considering the environmental impact of a major industrial project without the advantage of a full toxicological appraisal could well lead to inadequate protection of the aquatic environment due to insufficient lead-time for investigations. On the other hand, without the use of the 'twice-through' decision-pathway outlined in Figure 1, it is possible for costly 'over-protection' to be imposed by the courts due to public controversy over the absence of toxicological information regarding a critical waste component.

Under the terms of the National Development Act (1979), statutory authorities must prepare water right conditions irrespective of whether they consider that a waste should be discharged at levels or locations detailed in the application. Planning Tribunals presently have no power to reconvene after the initial enquiry in order to consider completed toxicological evaluations and confirm or adjust discharge recommendations.

Following the Planning Tribunal hearing, initial investigations have been undertaken regarding the toxicity of the biocide Alfloc 324 (equivalent to Nalco 7330).

Over-protection or under-protection: the once-through dilemma: Alfloc 324 contains 5-chloro-2-methyl-4-isothiazolin-3-one (1.15 per cent solution), and 2-methyl-4-isothiazolin-3-one (0.25 per cent solution). It functions as a broad-spectrum, non-oxidizing microbiocide, and is degraded by chemical, photochemical, and biological mechanisms. According to the manufacturer the principal degradation pathway involves hydrolysis and loss of chlorine and sulphur atoms to n-methylmalonamic acid. Degradation then proceeds through malonamic acid, malonic acid, acetic acid and formic acid, and finally to carbon dioxide.

Table 12 SUMMARISED LC50 AND LT50 TEST CONDITION INFORMATION FOR NAUPLII OF *ELMINIUS MODESTUS* EXPOSED TO ALFLOC 324

Each test number corresponds with that in Table 13

Test No.	Toxin	Mean temperature ture (°C)	Exposure time (hours)	Alkalinity (g/m^3 $CaCO_3$)	Salinity (ppt)	pH
67	Alfloc 324	13.0	1	117	31.1	8.1
68	Alfloc 324	13.0	2	117	31.1	8.1
69	Alfloc 324	13.0	3	117	31.1	8.1
70	Alfloc 324	13.0	-	117	31.1	8.1
71	Alfloc 324	13.0	-	117	31.1	8.1
72	Alfloc 324	13.0	-	117	31.1	8.1

Table 13 SUMMARY OF LC50 RESULTS AND LT50 RESULTS FOR NAUPLII OF *ELMINIUS MODESTUS* EXPOSED TO ALFLOC 324

Regression equation: $y = A + B\log_{10}x$

Test No.	Toxin	Exposure time (hours)	LC50 (95% CI) (ml/m^3)	Intercept	Slope	Variance of the slope	Probability
67	Alfloc 324	1	624 (369-1056)	3.54	0.52	0.001	0.0000
68	Alfloc 324	2	117 (82-165)	3.93	0.52	0.0009	0.0000
69	Alfloc 324	3	31 (23-41)	4.21	0.52	0.0009	0.0000

Test No.	Toxin (concentration ml/m^3)	LT50 (95% CI) (minutes)	Intercept	Slope	Variance of the slope	Probability
70	Alfloc 324 (10)	236 (81-690)	0.30	1.98	0.04	0.67
71	Alfloc 324 (30)	194 (83-451)	0.78	1.84	0.03	0.17
72	Alfloc 324 (100)	71 (36-139)	1.02	2.15	0.04	0.04

Table 14 SUMMARISED LC50 AND LT50 TEST CONDITION INFORMATION FOR NAUPLII OF *ELMINIUS MODESTUS* EXPOSED TO ZINC

Each test number corresponds with that in Table 15

Test No.	Toxin	Mean temperature ture (°C)	Exposure time (hours)	Alkalinity (g/m³ $CaCO_3$)	Salinity (ppt)	pH
73	Zn^{2+} as $ZnSO_4.7H_2O$	11.0	1	117	31.1	8.2
74	Zn^{2+} as $ZnSO_4.7H_2O$	11.0	2	117	31.1	8.2
75	Zn^{2+} as $ZnSO_4.7H_2O$	11.0	3	117	31.1	8.2
76-80	Zn^{2+} as $ZnSO_4.7H_2O$	11.0	-	117	31.1	8.2

Table 15 SUMMARY OF LC50 RESULTS AND LT50 RESULTS FOR NAUPLII OF *ELMINIUS MODESTUS* EXPOSED TO ZINC

Regression equation: $y = A + B\log_{10}x$

Test No.	Toxin	Exposure time (hours)	LC50 (95% CI) (mg/L)	Intercept	Slope	Variance of the slope	Probability
73	Zn^{2+} as $ZnSO_4.7H_2O$	1	27 (22-35)	3.17	1.27	0.01	0.003
74	Zn^{2+} as $ZnSO_4.7H_2O$	2	9 (7-10)	3.50	1.59	0.001	0.02
75	Zn^{2+} as $ZnSO_4.7H_2O$	3	5 (4-7)	3.93	1.48	0.01	0.0000

Test No.	Toxin (concentration mg/L)	LT50 (95% CI) (minutes)	Intercept	Slope	Variance of the slope	Probability
76	Zn^{2+} as $ZnSO_4$	11% mortality after 3 hours	(n=56)			
77	Zn^{2+} as $ZnSO_4$	22% mortality after 3 hours	(n=149)			
78	Zn^{2+} as $ZnSO_4$	112 (43-293)	0.005	2.44	0.17	0.28
79	Zn^{2+} as $ZnSO_4$	94 (43-208)	-1.74	3.41	0.22	0.08
80	Zn^{2+} as $ZnSO_4$	56 (22-141)	1.48	2.02	0.04	0.23

Table 16 BYSSAL THREAD GROWTH AND ATTACHMENT IN *PERNA CANALICULUS*

Control versus Alfloc 324-exposed mussels

		Concentration ml/m^3 Alfloc 324							
	Control	0.1	0.3	0.6	1.0	2.0	3.0	10.0	30.0
Number of mussels	40	30	30	30	30	30	30	30	30
Average length of byssal threads (±95% CI) (mm)	17.7±1.7 n=283	18.97±3.6 n=219	15.71±1.7 n=165	17.8±1.7 n=240	16.6±0.6 n=241	18.0±0.6 n=209	17.6±0.6 n=158	16.3±2.7 n=209	18.0±1.1 n=233
Number of threads/mussel	7.9±1.3	7.2 ±4.2	5.5±2.0	7.4±3.3	8.4±1.5	7.0±1.3	7.4±1.3	7.1±0.2	8.0±1.0
Average number of successful byssal thread attachments/mussel	5.4±0.7	6.0 ±2.0	4.3±2.0	5.5±0.9	6.4±2.4	5.5±1.9	6.3±3.4	5.8±0.1	6.6±1.0
Foot response: 0	1	0	3	0	0	0	3	0	1
1	14	12	10	8	8	19	17	20	11
2	25	18	17	22	12	11	10	10	18

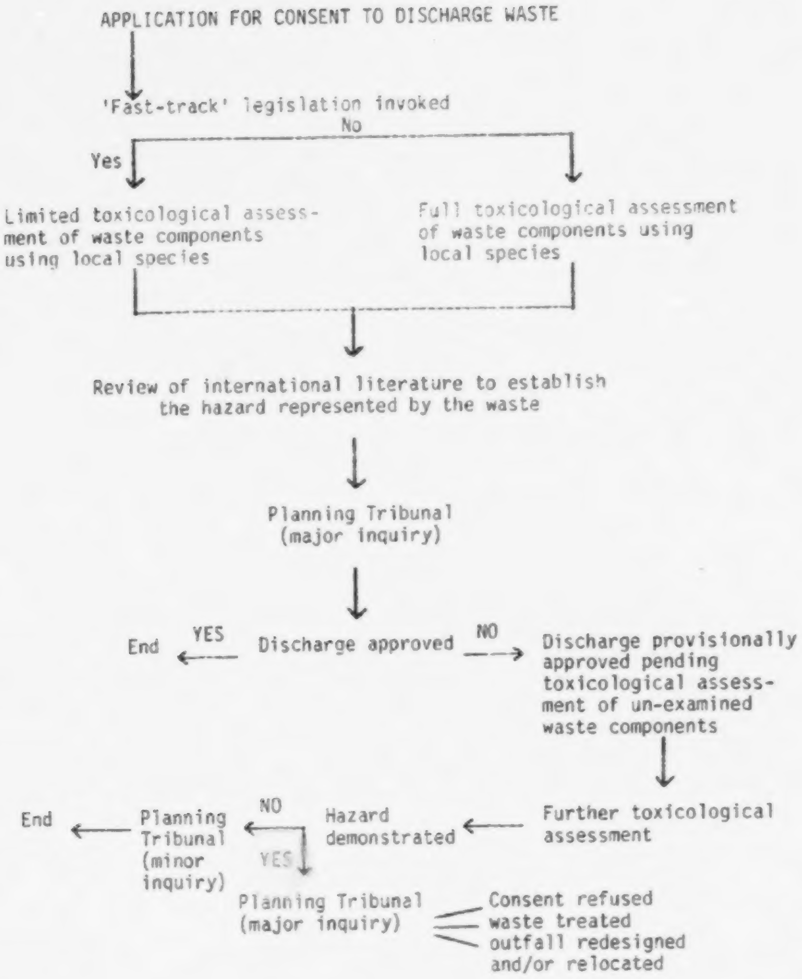
APPLICATION FOR CONSENT TO DISCHARGE WASTE
'Fast-track' legislation invoked
No
Yes
Limited toxicological assessment of waste components using local species
Full toxicological assessment of waste components using local species
Review of international literature to establish the hazard represented by the waste
Planning Tribunal (major inquiry)
End
YES
Discharge approved
NO
Discharge provisionally approved pending toxicological assessment of un-examined waste components
Further toxicological assessment
Hazard demonstrated
NO
YES
End
Planning Tribunal (minor inquiry)
Planning Tribunal (major inquiry)
Consent refused
waste treated
outfall redesigned and/or relocated

Figure 1 OPTIMAL DECISION PATHWAY UNDER FAST-TRACK LEGISLATION. THE 'TWICE-THROUGH' PATHWAY SHOWN ALLOWS REASSESSMENT OF WASTE DISCHARGE OPTIONS

When *P. canaliculus* were exposed to Alfloc 324 at concentrations up to 30 ml/m^3 for four weeks ($13.23+2.57^{o}C$; pH = 8.1-8.2; alkalinity = 114-119 g/m^3 $CaCO_3$, dissolved oxygen = 7.72 ± 1.71 ppm); filtering activity remained unchanged, clumping behaviour was not inhibited; byssal thread production and attachment were normal; and no mortalities occurred (Table 16). It is apparent from these results that zinc is many times more toxic to *P. canaliculus* than the biocide.

LARVAL STUDIES

Acute bioassays

The conditions and results of 1, 2 and 3 hr-LC50 tests and 10, 30 and 300 ml/m^3 LT50 tests of Alfloc 324 toxicity to nauplii of *Elminius modestus* are given in Tables 12 and 13.

The nauplii which collected in the bottom fractions during exposure to the biocide were not only those that were killed, but also those that were weakened and rendered unable to maintain their positive phototactic response. Such a sublethal effect is of obvious disadvantage to nauplii that must remain in the surface layer of the sea in order to successfully settle in their natural habitat.

The results of tests involving adult mussels and barnacle nauplii indicate that Alfloc 324 is not highly toxic to either larval or adult stages of marine invertebrates.

In order to compare the relative toxicities of Alfloc 324 and zinc to nauplii, tests were performed on *E. modestus* nauplii exposed to zinc. The conditions and results of 1, 2 and 3 hr-LC50 tests and 1, 3, 5, 10 and 30 mg/L LT50 tests of Zn^{2+} (as $ZnSO_4.7H_2O$) toxicity to nauplii of *E. modestus* are given in Tables 14 and 15. These results show zinc to be 23, 13 and 6 times more toxic to *E. modestus* napulii over 1, 2 and 3 hr exposures respectively.

Conclusion: It will rarely be possible for full toxicological hazard assessment to be compressed into the brief time available for investigations under fast-track legislation such as that recently introduced in New Zealand. Experience gained while processing the application for a right to discharge liquid wastes from the New Zealand Synthetic Fuels Corporation Limited's synthetic petrol plant at Motunui has highlighted the need for provision of a 'twice through' consent hearing process. A 'twice through' appraisal process would help to avoid either:

(a) under-protection of the aquatic environment; or

(b) unnecessary and often costly 'over-protection'.

ACKNOWLEDGEMENTS

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A PRELIMINARY EXAMINATION OF THE EFFECTS OF ETHYLENE DICHLORIDE ON THE HATCHABILITY OF COHO SALMON EGGS (ONCORHYNCHUS KISUTCH)

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REID, Bruce J., J.D. MORGAN and M.A. WHELEN. 1982. A preliminary examination of the effects of ethylene dichloride on the hatchability of coho salmon eggs (*Oncorhynchus kisutch*). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The effect of ethylene dichloride (1, 2 dichloroethane) on eyed coho salmon eggs (*Oncorhynchus kisutch*) was examined. Laboratory studies were conducted at 3° C in darkness using 21-day continuous exposure static replacement, and 1-hour slug dose bioassays. Eggs exposed to a slug dose of 100% ethylene dichloride for 1 hour died within 8 hours of exposure. Continuous exposure of eggs to 320 and 560 ppm ethylene dichloride resulted in 100% egg mortality within 8-9 days while partial mortality (46% average) occurred in the 150 ppm concentration over 21 days. Successful hatch (96% average) occurred in 56 ppm ethylene dichloride, but was severely reduced in 150 ppm (7% average) where the majority of the embryos died upon hatching. Alevins exposed to concentrations of 56 and 150 ppm ethylene dichloride died within 9 and 6 days, respectively of hatching. Results indicate that eyed coho eggs are less sensitive to ethylene dichloride than hatched alevins and that concentrations of less than 56 ppm would be required to obtain successful embryo to juvenile survival during continuous exposure to the chemical.

Key Words: Coho salmon, *Oncorhynchus kisutch*, ethylene dichloride, egg mortality, alevins, juvenile survival.

REID, Bruce J., J.D. MORGAN and M.A. WHELEN. 1982. Examen preliminaire des effets du bichlorure d'ethylene sur l'eclosion des oeufs du saumon coho (*Oncorhynchus kisutch*). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'examen porte sur les effets du bichlorure d'éthylène (1,2 bichloroéthane) sur les oeufs de saumon au stade où les yeux sont visibles. L'étude est faite en laboratoire, à la noirceur, à une température de 3° C pendant une période de 21 jours, par la méthode d'exposition continue et de remplacement statique, avec des tests de dosage létal de 1 heure. Les oeufs exposés à une dose létale de bichlorure d'éthylène meurent en moins de 8 heures. L'exposition des oeufs à une solution à teneur de 320 à 360 ppm en bichlorure d'éthylène entraîne la mort de 100 p. 100 des spéciments en moins de 9 jours, et à 150 ppm, entraîne la mort d'une partie des spécimens (46 p. 100 en moyenne) sur une période de 21 jours. Dans un bain à teneur de 56 ppm de bichlorure d'éthylène, l'éclosion se produit dans environ 96 p. 100 des cas; dans un bain à teneur de 150 ppm, l'éclosion est gravement réduite (jusqu'à 7 p. 100 en moyenne) car la majorité des embryons périssent au moment de l'éclosion. Les alevins exposés à des solutions d'une teneur de 56 ppm et de 150 ppm meurent, respectivement, 9 et 6 jours après l'éclosion. Les résultats semblent indiquer que les oeufs du saumon coho au stade où les yeux sont visibles sont moins sensibles au bichlorure d'éthylène que les jeunes alevins et que la concentration doit être inférieure à 56 ppm pour permettre la survie lors du passage du stade embryonnaire au stade d'alevin dans le cas d'une exposition continue au produit.

INTRODUCTION

Ethylene dichloride (1, 2 dichloroethane) is a colorless oily liquid used as a solvent and in the production of vinyl chlorides, paints, floatation reagents and scouring compounds (Hawley, 1977). Information concerning the toxicity of this compound to aquatic biota is mainly limited to fish. The 96-h LC50 values after exposures to ethylene dichloride range from 150 to 336 ppm for juvenile rainbow trout (R. Watts, pers. comm.; Bartlett, 1979) and from 150 to 175 ppm for marine pinfish (McKee and Wolfe, 1963). Recent studies using previously unexposed sockeye salmon alevins and fingerlings (Oncorhynchus nerka), pink salmon fry (O. gorbuscha) and water fleas (Daphnia pulex) resulted in 96-h LC50 values of 400, 235, 350 and 390 ppm, respectively (J.A. Servizi and D.W. Martens, pers. comm.).

On March 03, 1982 sixteen railcars containing ethylene dichloride overturned spilling approximately 500,000 L of the compound into the ice covered North Thompson River, B.C. Concern was expressed as to the effect of this chemical on water quality and fish species indigenous to the North Thompson River. This study was therefore initiated to determine the effects (if any) of ethylene dichloride on the eyed egg-to-fry survival of coho salmon (Oncorhynchus kisutch), a commercially important species which spawns in the mainstem North Thompson River and tributaries (Scott et al., 1982a; 1982b).

METHODS

COHO EGG ACQUISITION

Eyed coho salmon eggs (Oncorhynchus kisutch) were obtained from the Capilano River hatchery, North Vancouver, B.C. on March 29, 1982. Eggs were wrapped in cloth to minimize movement and physical damage and transported to the laboratory facilities in a water filled "Heath" incubation tray. The eggs were then transferred to a 38 L aquarium containing aerated dechlorinated tap water and acclimated in darkness for 48 hours at 3°C prior to testing.

TEST TOXICANT - ETHYLENE DICHLORIDE (1, 2 DICHLOROETHANE)

Production grade ethylene dichloride similar to that spilled in the North Thompson River was obtained from Dow Chemical of Canada Ltd., North Vancouver, B.C. on March 30, 1982. The chemical was stored in sealed glass bottles at 3°C for the test duration.

EGG HATCHABILITY TESTS

Continuous Exposure Tests

Continuous exposure egg hatchability tests were conducted in 38 L glass aquaria filled with 20 L of solution. Serial dilutions of ethylene dichloride were prepared by withdrawing one litre of water from each aquarium and pipetting a predetermined volume of ethylene dichloride into the one litre sample. The solution was vigorously mixed and added to the remaining water to obtain the desired concentration. The test concentrations included 56, 150, 320, and 560 ppm and represented values greater and less than 96-h LC50 ethylene dichloride concentrations for juvenile rainbow trout (Ron Watts, pers. comm.). Controls consisted of dechlorinated city tap water. All tests were conducted in duplicate to allow for natural variation in hatchability. Test solutions were pH unadjusted and unaerated to minimize volatilization of the chemical. Dissolved oxygen and pH were measured at experiment initiation and completion. Tests were conducted at 3.0 ± 0.5°C in darkness to simulate natural conditions.

Fifty eyed coho eggs were placed in each tank in a 25 x 25 cm mesh covered incubation tray which was suspended 1.5 cm above the aquaria bottom to maximize exposure to the toxicant and facilitate mortality checks. In the 56 ppm and control groups the trays were removed after hatching had occurred to minimize possible damage to the alevins. Coho egg loading density for each test aquaria was maintained at ≤ 0.5 g/l. Test solutions were changed daily (80% replacement) to ensure exposure to fresh toxicant.

Egg mortality, hatchability and alevin survival were recorded daily. Egg mortality was based on lack of movement and total opaqueness of the embryo. Hatchability was based on the rupturing of the egg chorion by the embryo and subsequent alevin survival at time of hatch. Alevin mortality was determined by lack of body movement and heartbeat and by body rigidity and opaqueness. Sublethal effects including abnormal alevin swimming behaviour were also noted. Tests were conducted until hatching was completed in control tanks or eggs/alevins had all died in toxicant exposure tanks (21 days).

Slug Dose Tests

In addition to continuous exposure tests, a "slug dose" experiment was conducted to simulate "worse case" chemical spill conditions. Fifty eyed eggs were exposed to one litre of 100% ethylene dichloride for a one hour period in a sealed, one litre glass jar. Tests were as previously described except that the test solution was slightly aerated through a pasteur pipette placed through the lid of the jar to maintain dissolved oxygen levels. After one hour, the eggs were transferred to a 38 L glass aquarium filled with 20 L of dechlorinated tap water. Egg survival was recorded at 1, 2, 4, 8, 24, 48, 72 and 96 h. Tests were conducted in duplicate to determine natural survival variability.

CHEMICAL ANALYSIS

Water samples (25 ml) were removed at 0, 12 and 24 hours from 56, 150 and 560 ppm tanks and analysed to determine actual test concentrations of ethylene dichloride over the first 24 hours. Statistical differences between predicted and actual concentrations were determined using the student's "t" test. Samples were collected centrally and mid-depth in each tank, placed in 25 ml glass vials with teflon septums and analyzed by purge and trap method employing gas liquid chromatography with flame ionization detection. Distilled water blanks were analyzed concurrently with each sample.

RESULTS AND DISCUSSION

PHYSICAL AND CHEMICAL CHARACTERISTICS

Dissolved oxygen levels remained at saturation ($\geq$13.5 mg/L) in all aquaria for the test duration. Solution pH ranged from 5.3 (560 ppm) to 5.8 (controls) in the continuous exposure tests; pH of the 100% ethylene dichloride sample was 5.5.

Actual concentrations of ethylene dichloride over the first 24 hour period in continuous exposure tests are summarized in Table 1. Expected and actual concentrations of ethylene dichloride did not differ significantly ($p \geq 0.05$) throughout the 24 hour period, and volatilization of the chemical, therefore, was not substantial.

EGG SURVIVAL

Slug Dose Experiments

Eggs exposed to 100% ethylene dichloride (EDC) for 1 hour then placed in freshwater began dying within 4 hours; 100% mortality occurred 8 hours after initial exposure to the chemical. Results show that 100% EDC exposure times of less than 1 hour would be required to achieve survival and hatchability of coho eggs.

Continuous Exposure Tests

Egg mortality in 150, 320, and 560 ppm EDC began after 6-8 days exposure. One hundred percent mortality occurred in eggs exposed to 320 and 560 ppm after 9 and 8 days, respectively, while 46% egg mortality occurred after 21 days of continuous exposure to 150 ppm. Timing of hatch and numbers of egg survivors was consistent between replicates with the exception of 150 ppm. Egg mortalities did not occur in 56 ppm or controls.

Bartlett (1979) and Watts (pers. comm.) reported EDC 96-h LC50 values of 336 and 150 ppm, respectively, for juvenile rainbow trout which suggests that salmonid eggs may be less sensitive to EDC than the juvenile life stage. The fertilized egg of many fish species is well protected against pollutants by a gelatinous membrane which may act as a physical and/or chemical barrier (Rosenthal and Alderdice, 1976). For example, acute toxicity studies with tropolone, a wood extractive, and coho eggs, alevins and juveniles indicated that eyed eggs were the least sensitive life stage (Peters et al., 1976). Similarly, Klaverkamp et al. (1977) reported that rainbow trout eggs were the least sensitive stage when compared to alevins, juveniles and adults after exposure to Fenitrothion, an organic pesticide. Further studies should examine the effect of EDC on egg survival from fertilization through embryonic development.

EGG HATCHABILITY

Results of egg hatchability after exposure to ethylene dichloride are summarized in Table 2, Figures 1 and 2. A 100% viable hatch occurred in control tanks; hatching commenced after 4 days and peak hatch occurred 6-7 days following test initiation. A slight reduction to 96% viable hatch occurred in eggs exposed to 56 ppm, whereby an average of 4% of the embryos died immediately upon hatching. Eggs in 56 ppm began hatching after 2 days, while peak hatch occurred 3-5 days after exposure to the toxicant, 1-4 days earlier than in the control tanks (Figure 2). In 150 ppm the average percent viable hatch was reduced considerably to 7% and was complete within 7 days of the initial exposure, following which time the remaining embryos died immediately upon (47%) or prior to (46%) hatching. Peak hatch occurred 8-13 days after the initial exposure to 150 ppm. As previously mentioned, all eggs exposed to EDC concentrations of 320 ppm and 560 ppm died 8-9 days after initial exposure. No alevin emergences were observed in these test concentrations prior to egg death.

Results of previous studies with coho and Atlantic salmon show a reduction of viable hatch at 4.4 ppm polychlorinated biphenyl (Halter and Johnson, 1974) and 0.01-0.10 ppm cyanide (Leduc, 1978). Larsen et al. (1977) found chloride concentrations up to 47 ppb did not affect survival or hatchability of coho salmon eggs. Premature hatching as observed in 56 ppm EDC has been recorded in previous hatchability studies with coho, pike and herring eggs (Halter and Johnson, 1974; Rosenthal and Sperling, 1974; Westernhagen et al., 1975). A possible explanation for this response is an alteration of the egg surface due to the toxicant, decreasing the permeability of the chorion to oxygen thus inducing early hatching (Halter and Johnson, 1974). Reduced permeability may also be responsible for egg mortality prior to hatching. The response of larvae dying immediately after emerging their heads has been recorded by other authors (Leduc, 1978; Servizi and Martens, 1978). Servizi and Martens (1978) suggest this response is due to a toxic effect on enzymatic processes which promote softening of the egg capsule prior to hatching. Improper softening of the egg wall could inhibit complete alevin emergence with death resulting from suffocation or direct contact with the chemical.

ALEVIN SURVIVAL

Results of alevin survival are presented in Table 2. Alevin mortality did not occur in control tanks during the 21 day exposure. Within one week of hatching, alevins exposed to 56 ppm exhibited sublethal effects including lethargy and disequilibria. Following this period accelerated alevin mortality was observed; 100% mortality occurred 9 days after hatching. Alevins exposed to 150 ppm became lethargic immediately following hatching; 100% mortality occurred 5-6 days later. Since 100% alevin survival occurred in control tanks, all mortalities were directly attributable to the toxic effect(s) of ethylene dichloride.

Results of the present study are consistent with those previously conducted (Halter and Johnson, 1974; Klaverkamp et al., 1977; Peters et al., 1976), whereby the hatched alevin stage was found to be more sensitive to pollutants than the salmonid egg. The sublethal effects of ethylene dichloride on alevins (lethargy, disequilibria) were also observed by

Servizi and Martens (pers. comm.) while testing the effects of EDC on sockeye and pink salmon early life stages (alevins, fry, fingerlings) and have been reported for other organochlorine toxicants (Halter and Johnson, 1974; Mount, 1962).

The sensitivity of the coho alevins to ethylene dichloride is comparable to recently reported toxicity values for previously unexposed juvenile rainbow trout (96-h LC50 = 150 ppm; R. Watts, pers. comm.) and sockeye fingerlings (96-h LC50 = 235 ppm; Servizi and Martens, pers. comm.). The coho alevins were found to be more sensitive than reported EDC toxicity values for sockeye alevins, pink fry and the invertebrate Daphnia pulex with 96-h LC50's of 400, 350 and 390 ppm, respectively (Servizi and Martens, pers. comm.) and earlier reported values for juvenile rainbow trout (96-h LC50 = 336 ppm, Bartlett, 1979). The apparent variation in sensitivities could be attributed to the behaviour of ethylene dichloride when mixed with water (i.e. volatilization) and the type of tests conducted (static vs. 24 h replacement in the present study). The apparent increased sensitivity of coho compared to sockeye alevins and pink fry may also be attributed to the continuous exposure from the egg stage and toxicity due to bioaccumulation of EDC during the study period. Further studies should include the acute toxic effects on all early life stages of salmonids (egg to juvenile) after slug dose exposures to aid in impact assessment of chemical spills.

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TABLE 1

CONCENTRATION OF ETHYLENE DICHLORIDE OVER A 24 HOUR PERIOD DURING CONTINUOUS EXPOSURE TESTS

Time (hr)	Expected Concentration (ppm)	Actual Concentration (ppm)
0	56	54* (52-56)**
	150	100* (98-103)**
	560	375* (365-385)**
12	56	79* (79-80)**
	150	105
	560	534
24	56	73
	150	124
	560	539

* mean of duplicate analysis

** range

TABLE 2

SUMMARY OF EFFECTS OF ETHYLENE DICHLORIDE ON EYED COHO EGGS

Ethylene Dichloride Concentration		Test	Response and Comments
Expected	Actual (After 24 hrs)		
0 ppm (Control)	0	21 day static replacement egg → alevin bioassays	- 100% hatch - time to initial hatch = 4 days - time to peak hatch = 6-7 days - 100% alevin survival
56 ppm	73	21 day static replacement egg → alevin bioassays	- 100% hatch (96% live, 4% dead) - time to initial hatch = 2 days - time to peak hatch = 3-5 days - 100% alevin mortality 9 days after hatching
150 ppm	124	21 day static replacement egg → alevin bioassays	- 54% mean hatch (7% live, 47% dead) - time to initial hatch = 2 days - time to peak hatch = 3 days - 100% alevin mortality 6 days after hatching - 46% egg mortality after 21 days
320 ppm	n.d.	21 day static replacement egg → alevin bioassays	- 100% egg mortality after 9 days
560 ppm	539	21 day static replacement egg → alevin bioassays	- 100% egg mortality after 8 days
100%	n.d.	1 hr slug dose then freshwater	- 30% egg mortality 3 hours after 1 hr exposure to EDC - 100% egg mortality 7 hours after 1 hr exposure to EDC

n.d.= not determined

Figure 1. Mean percent coho egg hatch from eyed stage in various concentrations of ethylene dichloride.

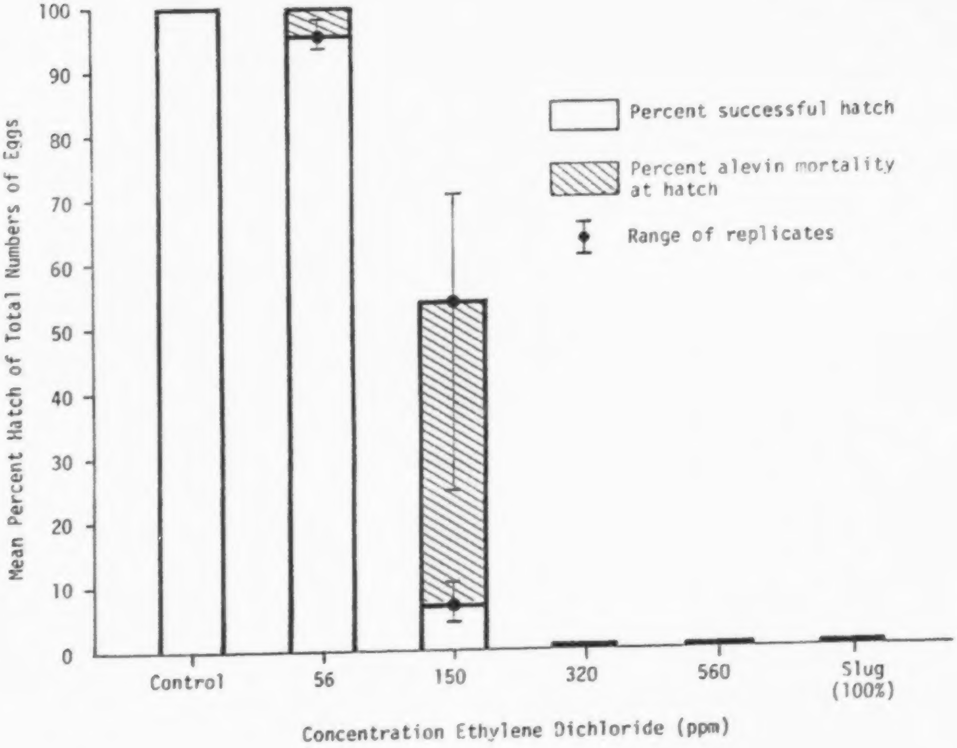
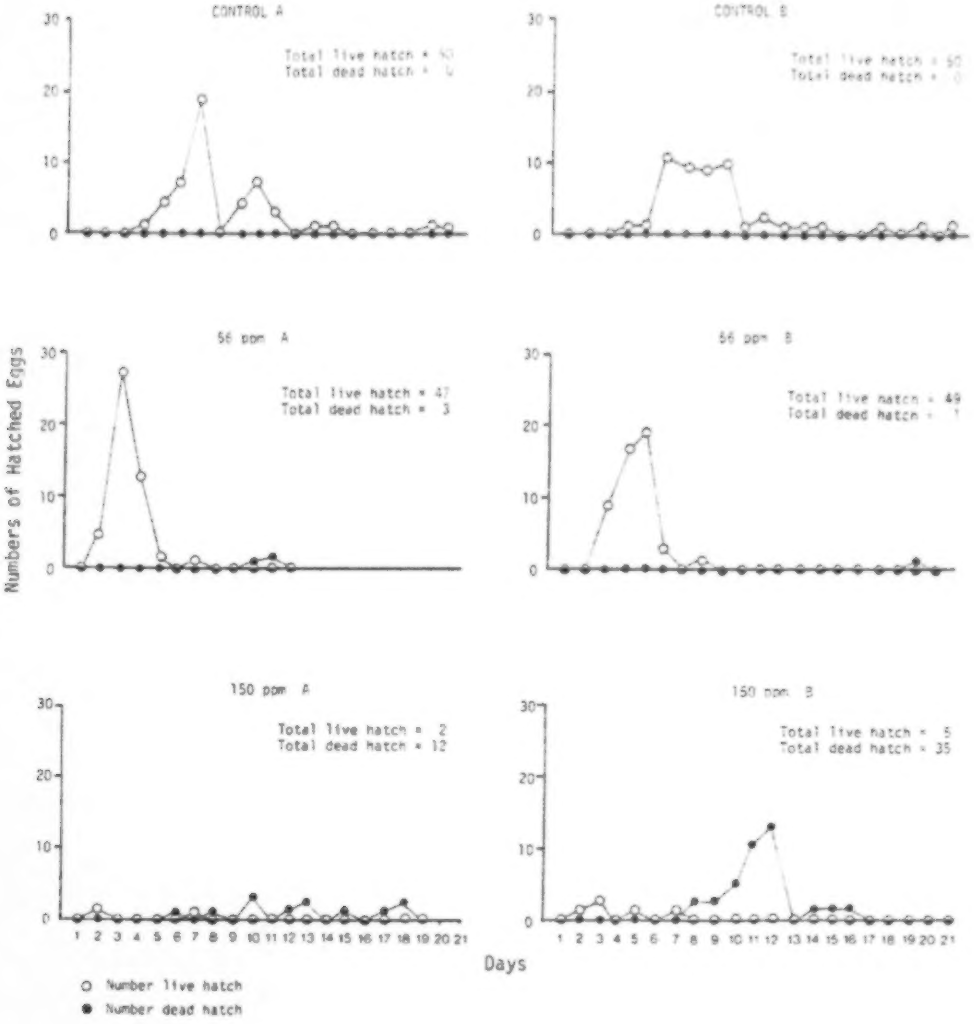


Figure 2. Daily hatch of coho eggs in various concentrations of ethylene dichloride.



EVALUATION OF LEVELS AND KINDS OF TOXIC EFFECTS ASSOCIATED WITH CHEMICAL FRACTIONS OF SEDIMENT FROM TOBIN LAKE, SASKATCHEWAN

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SAMOILOFF, M.R., E. ARNOTT, G.R.B. WEBSTER, J. BELL and D.A. BIRKHOLZ. 1982. Evaluation of levels and kinds of toxic effects associated with chemical fractions of sediment from Tobin Lake, Saskatchewan. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Two short-term biological tests, the *Salmonella typhimurium* (Ames) test for mutagenesis and the *Panagrelius redivivus* (nematode) developmental assay were used on chemical extracts of Tobin Lake, Saskatchewan, sediment samples. Two types of biological effect could be detected with *S. typhimurium*; (1) cytotoxic effects, limiting bacterial growth, and (2) mutagenesis. The *Panagrelius redivivus* developmental assay detects 4 types of effect (in decreasing order of priorized risk) : (1) lethality, (2) sublethality, (3) inhibition, (4) mutagenesis. The chemical extraction protocol involves both differential solubility and elutability from Florasil. Sites near the point where the Saskatchewan River enters the lake show maximal toxic effects in a neutral fraction eluting from florasil with 1:1 hexane-dichloromethane. Sites further downstream show toxicity associated with more polar fractions. Fifty-four potentially toxic compounds were identified in the toxic neutral fraction from the upstream sites although only 5 compounds were found in the toxic fraction from both upstream sites. These compounds are 2-nonenal, hexyl-3 methyl cyclopentane, 1,9-nonanediol, tetradecanol and benzaldehyde.

Key Words: *Salmonella typhimurium*, *Panagrelius redivivus*, cytotoxic effects, mutagenesis, Ames test, 2-nonenal, hexyl-3 methyl cyclopentane, 1,9-nonanediol, tetradecanol, benzaldehyde.

SAMOILOFF, M.R., E. ARNOTT, G.R.B. WEBSTER, J. BELL and D.A. BIRKHOLZ. 1982. Évaluation de la nature et de la gravité des effets toxiques associés aux fractions chimiques tirés de sédiments en provenance du lac Tobin (Saskatchewan)

Des extraits chimiques d'échantillons sédimentaires provenant du lac Tobin (Saskatchewan) sont soumis à deux analyses biologiques à court terme: le teste de mutagénèse de *Salmonella typhimurium* (Ames) et l'analyse de croissance du *Panagrellus redivivus* (nematode). Deux effets biologiques résultent du test de *Salmonella typhimurium*: 1) les effet cytotoxiques, limitant la croissance bactérienne et 2) la mutagénèse. La deuxième analyse a quatre effets, énumérés ici par ordre décroissant de gravité: 1) la letalité, 2) la sublétalité, 3) l'inhibition et, 4) la mutagénèse. La méthode d'extraction des substances chimiques se fonde à la fois sur les différences de solubilité et sur la possibilité d'élution à partir de florisil. Les sites se trouvant à proximité de l'embouchure de la Saskatchewan sont ceux où les effets toxiques sont les plus marqués dans une fraction neutre éluée à partir d'une colonne de florisil, le rapport d'hexane au dichlorométhane étant de 1/1. Aux sites d'aval, le niveau de toxicité est lié à des fractions plus polarisées. Cinquante-quatre composés à potentiel toxiques sont repérés dans la fraction toxique neutre tiree des sites d'amont, bien que seulement cinq d'entre eux, le nonanol-2, le hexyl-1 methyl-3 cyclopentane, le nonanédiol-1,9, le tétradécanol et le benzaldéhyde, se retrouvent dans la fraction toxique tirée des deux sites d'amont.

EVALUATION OF LEVELS AND KINDS OF TOXIC EFFECTS ASSOCIATED WITH CHEMICAL FRACTIONS OF SEDIMENT FROM TOBIN LAKE, SASKATCHEWAN

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INTRODUCTION

Any aquatic system in close proximity to human activity may contain literally thousands of contaminating chemicals and their by-products. A very limited number of these contaminating chemicals will have sufficient known toxic effects to be placed on a toxic chemicals list, and still fewer can be effectively controlled by existing legislation. The major problem in such contaminated systems is to determine and control those components of the contaminated system that pose the greatest risk to biological systems. Two methods of determining such risk are at present widely used. The first involves the determination of chemical species present in the system, and matching these chemicals to a previously established toxic chemicals list. The second method involves the *in situ* monitoring of specific indicator organisms in the contaminated environment to detect toxic effects of chronic exposure to the battery of contaminants. Both methods have strong advocates and opponents, and we will not comment further on these two approaches.

As a third method for determination of those agents contributing to risk, we have developed a protocol involving chemical fractionation of environmental samples, coupled with biological testing of these fractions. This protocol permits us to utilize the biological data to establish which components of the contaminated system pose the greatest risk.

This protocol involves: (1) fractionation on the basis of differential solubility of extracts from sediment samples from a contaminated aquatic ecosystem, (2) the bioassay of each fraction to ascertain the relative toxicity of each and (3) the identification of the components of the major toxic fraction. Fractionation of the original extract reduces the total number of contaminants tested in each fraction. The fraction showing greatest toxicity can be re-fractionated to again reduce the number of contaminants present, until the actual toxic materials can be identified.

Two bioassay methods are utilized. The *Salmonella typhimurium* test (1,2) is used to detect mutagenesis. The free-living nematode, *Panagrellus redivivus*, is used to detect lethal, semilethal, developmental, and genotoxic effects (3).

As material to perform preliminary studies of this protocol, we have selected sediment samples from Tobin Lake, on the Saskatchewan River, which is exposed to contaminants from agriculture, mining, petrochemical industries, pulp and paper mills, and municipalities. Tobin Lake represents a major sink for these contaminants. From this extensive range of sources of contamination, we are attempting to focus on those compounds that represent the greatest potential risk.

MATERIALS AND METHODS

SAMPLING PROGRAM

The initial studies reported here were made with sediment samples taken from Tobin Lake at sites designated Site A, Site B, Site C, and Site D.
Site A is near the upstream end of Tobin Lake, where the Saskatchewan River flows into the lake. The site designated Site B is situated approximately 12 km downstream from Site A. Site C is at approximately the mid-point of the lake. Site D is in the furthest downstream region of Tobin Lake. Site A represents the region of maximum flow of water into the lake, while Site D represents the region of minimal flow.

Sediment samples were collected by Eckman dredge, and temporarily held in polyethylene bags. These samples were quickly transferred to pre-cleaned tin-plated cans. Samples in the cans were frozen and stored at -40° C. prior to analysis .

CHEMICAL EXTRACTION PROCEDURES

Samples were thawed at room temperature, lyophilized, and then homogenized by placing in a soil roller for 4h. Each sample was divided into five replicate portions, each weighing approximately 40g. Each replicate was re-hydrated with organic-free water to raise its moisture content to 25% and then extracted following the protocol described below.

1. The rehydrated replicate was Soxhlet extracted in acetone-hexane (1:1) for 24h. The extracted sediment was retained for further extraction (point 5 below).

2. The acetone-hexane extract was preconcentrated and partitioned into organic-free water. The water was adjusted to pH 7.0 and extracted with dichloromethane. The aqueous phase was retained for further extraction (point 4 below).

3. The dichloromethane extract, containing neutral compounds, was dried by passage through a column of anhydrous sodium sulphate, preconcentrated, and the dichloromethane evaporated following addition of 10mL hexane. This hexane solution was applied to a 10g Florisil column (5% water deactivated).

a) Material eluted from the Florisil column by 160mL hexane was termed Fraction 1.

b) Material eluted from the Florisil column by 160mL 1:1 hexane-dichloromethane was designated Fraction 2.

c) Material eluted from the Florisil column by 200mL dichloromethane was designated Fraction 3.

d) Material eluted from the Florisil column by 200mL methanol was designated Fraction 4.

4. The aqueous phase of the original dichloromethane extraction (Step 2) was further fractionated as follows:

a) The aqueous phase was adjusted to pH greater than 11 by addition of 6M sodium hydroxide, and extracted with dichloromethane. The dichloromethane was removed and the extracted material, containing strong bases, was designated Fraction 5.

b) The aqueous phase was adjusted to pH less than 2 by addition of 6N sulfuric acid, and extracted with dichloromethane. The dichloromethane was removed from the extract. This extract, containing strong acids, was designated Fraction 6.

5. The extracted sediment (point 1) was extracted with methanol for 24h. This methanol extract was termed Fraction 7.

6. The remainder of the aqueous extract was returned to pH 7.0, and designated Fraction 8, the hydrophilic compounds.

Fractions 2-7 were exchanged into dimethylsulfoxide for biological testing. Extracts were diluted 1:1 in DMSO and evaporated at 70° C until only the volume of DMSO remained. Fraction 1 was tested in hexane, while the aqueous Fraction 8 was tested as a water extract.

THE Panagrellus redivivus ASSAY

Detailed descriptions of culture media and methods for the *P. redivivus* assay have been previously presented (3). In brief, the test involves exposing a synchronously growing population of second stage juvenile nematodes to the test extract for a 96h growth period, and comparing the growth and survival of these animals to control populations. There are four post-embryonic stages (L2, L3, L4 and adult), each characterized by a specific size range. Growth from one stage to the next requires normal physiological and informational processes; growth will be inhibited if these processes are blocked or inhibited. The stage distribution of test and control animals represents the primary set of data from which toxicity is determined. The stage distribution can be analyzed to provide evidence for four distinct toxicological effects:

1. Lethality, in which 100% of the tested population does not survive the growth period, with no significant decrease in the survival of the control population.

2. Semilethality, in which a significant proportion of the test population dies, while the control population has no significant death.

3. Inhibition, in which the proportion of growth to each stage is inhibited relative to controls. This effect indicates that exposure to the tested material is inhibitory or debilitating to normal physiological processes.

4. Genotoxicity, which is manifested as the specific inhibition of the molt to the adult stage, relative to controls. Growth through the final molt requires extensive gene expression, and this growth is highly sensitive to known mutagens. In this context, genotoxicity refers to blockage in the utilization of genetic information, either via mutagenesis or inhibition of RNA or protein synthesis.

The degree of toxic effects produced by different fractions can be determined by the extent to which the test population distribution varies from the control population distribution. One method of expressing this deviation is by calculating a 4X2 chi-square value representing the stage distributions of test and control populations. The chi-square value is used as a scalar quantity, with greater values representing increased toxicity.

Blind coded Tobin Lake extracts, and identified controls containing the appropriate solvents exchanged into appropriate carriers, were diluted 1/126 in M9 phosphate buffer (4) containing 0.5μg/mL cholesterol and

5mg/mL autoclaved yeast. This diluted test or control extract was used as *P. redivivus* growth medium. A total of 5 tests, each consisting of 10 replicates of 10 animals each, were performed for each tested fraction.

THE *Salmonella typhimurium* ASSAY

Tests are performed using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, each strain being used both with and without S9 activation (2). The S9 was obtained from homogenates of liver from rats activated by Arochlor. The addition of S9 in the *S. typhimurium* assay detects the metabolic conversion of a promutagen to a mutagenic substance. Fractions 2-7 were diluted 1:8 in dimethylsulfoxide for testing. Fraction 1 was diluted 1:8 in hexane, while Fraction 8 was tested undiluted. Each diluted extract was applied to a test plate in 20µL, 50µL, 100µL, or 200µL aliquots by the plate incorporation method. Preliminary studies showed that 100µL aliquots of DMSO had no detectable genotoxic or cytotoxic effects.

Two types of responses to the extracts were determined. The criterion used in this study for genotoxicity of a particular extract was a minimum of a three-fold increase in revertant colonies at a high concentration of extract, or in two successive concentrations. Inhibition was indicated by either a reduction in revertants at higher concentration or as a reduction in the cell lawn density as indicated by microscopic examination.

RESULTS

THE *P. redivivus* ASSAY

The nematode assay indicates that the most toxic components in Tobin Lake sediments are associated with the further upstream sites (Table I). At the upstream sites, Fraction 2 is the most toxic. This component is semilethal in the sediments from the furthest upstream sample site (Site A), producing 83% mortality in 96 hours (chi square=268). Further downstream, at Site B, Fraction 2 is still the most toxic, but at this site Fraction 2 is inhibitory (chi-square=360), with only 26% mortality after 96 hours. At the upstream sites, the water soluble fraction (Fraction 8) is the second most toxic fraction, producing developmental inhibition with a chi-square value of approximately 160 at both upstream sites. Fractions 3 and 7 also produce highly significant developmental inhibition at the upstream sites. By the mid region of the lake (Site C), and further downstream (Site D), Fractions 2, 8, 7 and 3 had no detectable toxic effects.

The changing pattern of toxic effect with position in the lake is shown by the following ranking of the toxic effects by fraction number at the sample sites (moving from upstream to downstream sites):

SITE A: 2>8>3>7
SITE B: 2>8>7>3
SITE C: 4
SITE D: 5>4>1

The total toxicity found at Sites C and D was much lower than that found at the upstream sites.

THE *S. typhimurium* ASSAY

The Ames test was performed on fractions obtained from Sites A and B sediments. The *S. typhimurium* assay (Table II) established Fraction 2 as the most toxic of the fractions from these sites. Fraction 2 from Site A inhibited growth in four of the five test strains, although this inhibition was released in one of the strains by addition of S9. Direct-acting mutagenesis (mutagenesis

TABLE I.

The _P. redivivus_ assay of Tobin Lake extracts.

	SITE A	SITE B	SITE C	SITE D
FRACTION 1	-	-	-	G (26)
FRACTION 2	S (268)	I (380)	-	-
FRACTION 3	I (102)	I (68)	-	-
FRACTION 4	G (16)	-	I (23)	I (18)
FRACTION 5	-	G (16)	-	I (38)
FRACTION 6	-	G (25)	-	-
FRACTION 7	G (99)	I (87)		
FRACTION 8	I (161)	I (164)	-	-

S=Semilethal I=Inhibition of development G=Genotoxic
Numbers in parenthesis indicate chi-square value of test population distribution compared to control population distribution.

TABLE II.

Salmonella Typhimurium assays of Tobin Lake fractions.

		FRACTION NUMBER AND EFFECT															
		F1		F2		F3		F4		F5		F6		F7		F8	
STRAIN		I	M	I	M	I	M	I	M	I	M	I	M	I	M	I	M
SITE A																	
TA1535		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1535	+S9	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1537		-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
TA1537	+S9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TA1538		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1538	+S9	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
TA98		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
TA98	+S9	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA100		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA100	+S9	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
SITE B																	
TA1535		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
TA1535	+S9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TA1537		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1537	+S9	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1538		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA1538	+S9	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+
TA98		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA98	+S9	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA100		-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
TA100	+S9	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Column I indicates inhibition produced by a particular fraction.
Column M indicates mutagenesis detected in a particular fraction.

without the addition of S9) was observed in three of the five test strains using Fraction 2 from Site A, while one strain indicated a promutagen (mutagenic only in presence of S9) in this fraction. Inhibition of growth produced by Fraction 8 from Site A was also observed in one of the five strains following S9 activation. Fraction 7 from Site A was mutagenic to one strain without S9 activation, and to one strain following S9 activation.

Growth of all five strains was inhibited by Fraction 2 from Site B, although in two of the test strains this inhibition was removed by the addition of the S9 extract. Four of the five test strains showed direct-acting mutagenic activity of Fraction 2 from Site B, and in only one strain was the mutagenesis eliminated by addition of S9. Fraction 8 from Site B produced growth inhibition in one of the five strains following S9 activation, and Fraction 3 from Site B was mutagenic to this strain following S9 activation.

PRELIMINARY ANALYSIS OF FRACTION 2

On the basis of the above results, samples of Fraction 2 from Sites A and B were subjected to chemical analysis by gas chromatography-mass spectroscopy. A total of 30 and 27 potentially toxic compounds were identified in Fraction 2 from sites A and B, respectively (Table III). Five compounds were common to Fraction 2 of both sites. These common compounds were: 2-nonenal, hexyl-3-methyl cyclopentane, 1,9-nonanediol, tetradecanal and benzaldehyde.

DISCUSSION

The approach for determining the extent and nature of contamination of aquatic ecosystems outlined in this presentation depends upon the validity of the biological assays utilized. The *P. redivivus* assay is rapid, cost-effective and sensitive, although the nematode assay has not been as extensively utilized as the *S. typhimurium* test. A comparison between the Ames and *P. redivivus* test is shown in Table IV.

In all cases where the Ames test found inhibition or mutagenicity, the *P. redivivus* assay also detected inhibition or genotoxicity. The *P. redivivus* assay detected low level genotoxicity or inhibition in 4 of the 10 fractions in which the Ames test found no inhibition or mutagenicity.

The *P. redivivus* bioassay system gives comparable results to the *S. typhimurium* tests, showing somewhat greater sensitivity.

Both the Ames and nematode test provide convincing evidence that the major toxic components of the upstream sediment extract reside in Fraction 2, with Fraction 8 the second most toxic component. Fraction 3 is the third most toxic component. Fraction 2 represents those neutral compounds soluble in 1:1 hexane-dichloromethane. There is little published information on many of the 52 potentially toxic compounds found in Fraction 2, and this fraction might not, *a priori*, contain the most highly priorized chemicals present in the sediments. Ethenyl benzene (styrene) has been shown to be mutagenic to *S.typhimurium* both with and without S9 activation (5). Styrene is also cytotoxic to *S. typhimurium* (6,7), which may account for some of the observed inhibition. The styrene metabolite, benzyl alcohol, is also mutagenic (8), and this compound is similar to benzaldehyde. Benzaldehyde was not mutagenic, as determined by the Ames test, although 3 of 19 related aldehydes tested were mutagenic (9).

Twenty-five identified Fraction 2 compounds are unique to Site A , while 22 identified Fraction 2 compounds are unique to Site B, with only five

TABLE III.

COMPOUNDS TENTATIVELY IDENTIFIED IN FRACTION 2 EXTRACTS

SITE A	SITE B
benzaldehyde	benzaldehyde
1-hexyl-3-methyl cyclopentane	1-hexyl-3-methylcyclopentane
tetradecanal	tetradecanal
1,9-nonanediole	1,9-nonanediole
2-nonenal	2-nonenal
cis-1,2-diethyl cyclobutane	4-hydroxy-4-methyl-2-pentanone
2,3,3-trimethyl-1-butene	4-hydroxy-5-methyl-2-hexanone
2-methyl-3-pentanol	2-methyl-3-pentanol
2-hexanethiol	1-phenyl ethanone
trans-2-methyl-cyclopentanol	2-hexanethiol
ethenyl benzene (styrene)	1-ethoxy-2-heptanone
diethyl phthalate	dihydro-5,5-dimethyl-2(3H)-furanone
o-neo-isomenthol	2-ethyl-1-hexanol
6,10,14-trimethyl-2-pentadecanone	3,3-dimethyl hexanal
citronellyl propionate	2-methyl benzaldehyde
13-octadecanal	3- or 4-methyl benzaldehyde
7-butyl-bicyclo(4.1.0)heptane	3-propoxy-1-propene
1-doctriacontanol	3,5,-dimethyl-cyclohexen-1-one
17-octadecanal	5-octadecanal
2-dimethyl benzene	4-decanone
4-methyl hexanal	1,1,3-trimethyl cyclopentane
1-hexene-3-ol	*iso*-octanol
6-methyl-2-heptanone	cyclododecane
3,3-dimethylhexane	3,7-dimethyl-6-octen-1-ol
2,2,3,4-tetramethyl pentane	2-methyl-1-dodecanol
nonadecanol	cholesterol
1,12-tridecadiene	gamma-sitosterol
hexadecanol	
campesterol	
3-methyl-2-cyclohexene-1-one	

TABLE IV.

Comparison of bacterial and nematode bioassays on fractions of Tobin Lake sediments.

		Bacterial Test		Nematode Test	
	FRACTION	Effect	Rank	Effect	Rank
Site A	1	-	-	-	-
Site A	2	I, M	1	SL	1
Site A	3	-	-	I	3
Site A	4	-	-	M	5
Site A	5	-	-	-	-
Site A	6	-	-	-	-
Site A	7	M	3	M	4
Site A	8	I, M	2	I	2
Site B	1	-	-	-	-
Site B	2	I, M	1	I	1
Site B	3	M	3	I	4
Site B	4	-	-	-	-
Site B	5	-	-	M	6
Site B	6	-	-	M	5
Site B	7	-	-	I	3
Site B	8	I, M	2	I	2

M=genotoxic I=inhibitory SL=semilethal -= no detected effect
The effects are ranked with the most toxic fraction being assigned the lowest number (highest priority).

Fraction 2 compounds common to the two sites, although the sites are separated by only 12 km on the same river system. This difference in composition may account for the different toxicological effects observed between Site A and B Fraction 2 extracts.

The results reported here demonstrate that the classes of chemicals in environmental samples can be evaluated and ranked on the basis of their relative toxic effects. Although the work reported here does not provide the identity of the individual most toxic compounds present, it does identify the class of compounds producing the greatest risk. Furthermore, it significantly reduces the number of compounds considered as the highest priority contaminants of these two sites on Tobin Lake. The lack of detectable toxicity in Fraction 1 argues against serious contamination at these sites by pesticides, which would be contained in this fraction. That pesticides are not the major risk producing chemicals is further supported by the lack of detectable levels of pesticides in Fraction 2. Similarly, one of the major toxic products of pulp mills, diterpene resins, would be present in Fraction 6, which is only the fifth most toxic fraction (slightly genotoxic) at Site B, and not toxic at the other sites. This does not argue that such compounds are not present, but suggests that they do not represent the most important sources of risk at these sites.

The relative ranking of toxic classes (i.e, Fractions 1-8) can be examined spatially within the ecosystem studied. The greatest toxic effects are localized at the upstream end of the lake, with greatly reduced toxic effects at the downstream end.

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WATER TREATMENT FOR FOULING PREVENTION. RESIDUAL TOXICITY OF AN ORGANO - TIN COMPOUND TOWARDS MARINE ORGANISMS

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SAROGLIA, Marco G., Romano AMBROGI and Gioacchino SCARANO. 1982. Water treatment for fouling prevention. Residual toxicity of an organo - tin compound towards marine organisms. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Acute toxicity of an aliphatic organo-tin compound utilizable in anti-fouling treatment has been tested towards 5 marine species. A bioassay to analyse the active concentration of the biocide has been developed by using *Brachionus plicatilis* and *Artemia salina*. The fate of the compound in the marine environment and the possible impact to the ecosystem due to a continuous discharge of tin in a marine water body are discussed.

Key Words: Toxicity, marine organisms, organo-tin, *Brachionus plicatilis*, *Artemia salina*, tin.

SAROGLIA, Marco G., Romano AMBROGI and Gioacchino SCARANO. 1982. Traitement préventif contre la salissure -- Toxicité résiduelle d'un composé organostannique pour les organismes marins. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On vérifie la toxicité aiguë, pour cinq espèces marines, d'un composé organostannique acyclique pouvant servir au traitement préventif contre la salissure. Se servant de spécimens de *Brachionus plicatilis* et d'*Artemia salina* les chercheurs mettent au point un essai biologique permettant d'analyser la concentration active de ce biocide. Ils discutent également de l'evolution du composé en milieu marin et des répercussions possibles sur l'écosystème du déversement continu d'étain dans la mer.

INTRODUCTION

The chemical treatment of once-through condenser cooling water (CCW) has always been a routine procedure at those electric power stations where biofouling has contributed to reduced condenser efficiency. Among chemical compounds employed as antifouling agents chlorine is the most widely used.

Since the discoveries by Rook (1974) and Bellar *et al*. (1974) that the chlorination of water containing organic matter produces trihalomethanes, more and more attention has been devoted to the possible formation of halo-organic compounds possibly dangerous for human health, in particular when high chlorine concentrations are needed for a useful antifouling treatment.

The available alternatives to chlorination are numerous but apparently none of them has received wide acceptance for reasons based either on economic or environmental considerations.

Biocides belonging to the organo-tin category have been largely utilized as components in antifouling paints and are now available for continous water treatment as well.

Among them a new compound is manufactured by "Borman Italiana", Settimo Milanese (Milano) and labelled as MA 1161. The compound is an organo-tin in which the metal is bonded to linear aliphatic hydrocarbon chains. Preliminary studies on the acute toxicity of this compound have been carried out by Mor (1978, 1979, and 1981) on *Mytilus galloprovincialis*, *Hydroides elegans* and *Mugil cephalus*. After preliminary evaluation MA 1161 was used in Italy, in plants utilizing marine water, as a continous antifouling treatment at a concentration of 0.05 mg/l plus two weeks/year with shock-treatment of 0.33 mg/l. The first plants utilizing the compound were 2 electric power stations, a petro-chemical plant and a steel foundry; all of them were located in areas characterized by heavy organic pollution in water. Results were declared very successful by plant operators, who had earlier experience with hypochlorite treatment.

In order to assess the acceptability of wider use of the product as a possible alternative to chlorination, we started a research programme comprising the study of the effectiveness, ecotoxicology and environmental impact of such organo-tin compounds.

While the environmental impact of chlorine as an antifouling agent is well documented, (see for instance Mattice and Zittel, 1976) studies on organo-tin compounds are rather scarce, (Smith,1978; Jensen,1977; Zuckerman, *et. al.* 1978), the problems of organo-tin ecotoxicity and the fate of organo-tin compounds in the aquatic environment remain largely unsolved. Our first objective was to assess acute toxicity and promote a discussion about the possible effects of organo-tin and tin discharges in the marine environment.

In this paper, some data are reported concerning the acute toxicity of MA 1161 to five species of marine organisms including algae, invertebrates and fish.

METHODS

Acute toxicological tests have been carried out by exposing algae, Rotifera, Copepoda and fish to biocide MA 1161.

Tests with algae

Cultures of the algae *Tetraselmis suecica*, at an initial density of 500,000 cells/ml, were prepared in 10L transparent atoxic politene bags. The medium was prepared from filtered sea-water, with nutrient added in algal culture for aquacultural purposes. Cultures were maintained at 22°C in continuous artificial illumination at 3000 lux and aerated gently.

Tests were run at 0.01, 0.05, 0.1, 0.2, 0.3, and 0.4 mg/L of MA 1161 plus control. Each concentration was replicated 3 times. At the starting time and after 24, 48, 72, 96, 168, 192, and 216 hours the actual number of cells per ml were counted under a microscope utilizing a Burker cell.

Tests with invertebrates

All tests with Rotifera, Copepoda and brine shrimps were run under the same basic conditions.

Coastal marine water at pH 8.2 and salinity 36% were utilized for dilution, after filtration through a 0.45 μm filter and oxygenation until saturation at 22° C.

Tests were run at 22° C and constant illumination in Petri dishes containing 10 mL of solution.

Mortality data were analysed with a computerized Probit Analysis program for evaluation of the 24 hours LC50.

Rotifera - One hundred *Brachionus plicatilis* were introduced into each Petri dish by collecting them with a Pasteur pipette from a basic culture growing on *Tetraselmis*.

Tests were run at 0.01, 0.03, 0.05, 0.07, 0.08, 0.1, 0.15, 0.2, 0.3, and 0.4 mg/L MA 1161 plus the control. Each concentration was replicated three times. The concentrations 0.01, 0.03, 0.05, 0.1, and 0.2 mg/L were repeated a second time, again with 3 replicates.

Mortality was observed under a stereo-microscope after 24 hours exposure. All those organisms showing no mortality after stimulation with a needle were considered dead.

Copepoda - One hundred adult Copepoda (*Tisbe furcata*) were introduced into each Petri dish, following the procedure utilized for Rotifera. Tests were run at 0.01, 0.03, 0.05, 0.08, 0.1, and 0.2 mg/L MA 1161 plus control. Each concentration was replicated 3 times.

Brine shrimp - A teaspoon of *Artemia salina* cystis were incubated for 24h at 25° C in a 200 ml tube filled with filtered marine water with continuous illumination and aeration. After 24 hours nauplii they were transferred to a second 200 ml glass tube and aerated gently. After succeeding 24 hours, 100 brine shrimp (nauplii II and metanauplii) were introduced into each dish at concentrations of 0.05; 0.1; 0.2; 0.3; 0.5; 0.7; 0.9; 1.2; 1.5; 1.7; and 2.0 ppm MA 1161 plus control each with 3 replicates.

Tests with fish

Tests were run in 40-litre glass aquariums. Ten seabass (*Dicentrarchus labrax*) 5 ± 0.5 cm long, standard length, were placed in an aquarium at each concentration. Test concentrations were 0.02, 0.05, 0.07, 0.09, 0.1, 0.3, and 0.5 mg/L MA 1161 plus control, prepared with filtered sea water. Each concentration was replicated twice. Solutions were replaced each 24 hours and aerated continuously. Tests were run at 24° C with natural light.

Mortality was checked at 1, 3, 6, 24, 48, 72, and 96 hours. Fish showing no reaction to caudal peduncle stimulation were considered dead. Ninety-six hour LC50 values were determined using Probit Analysis.

Results

An algal growth inhibition is suggested by Fig. 1 for concentrations greater than 0.2 mg/L. Such inhibition increased at 0.3 and 0.4 mg/L, particularly after 24 hours exposure. Later on, the algal growth rate rose again and the cell density reached values close to the control culture after 168 hours.

The 24 hours LC50 for invertebrates was calculated at 0.076, 0.086 and 1.27 mg/L respectively for *Brachionus plicatilis*, *Tisbe furcata* and *Artemia salina*, (Fig. 2). The 96 hours LC50 for fish (*Dicentrarchus labrax*) was 0.073 mg/L of MA 1161 (Fig.3). A mortality below 10% was observed in *Dicentrachus labrax* exposed for 96 hours at a biocide concentration of 0.05 mg/L.

DISCUSSION

The acutely toxic concentrations found in our tests for the biocide MA 1161 were for invertebrate and fish species 0.07 - 1.3 mg/L, the most resistant species being *A. salina*. Algal growth was affected only at 0.2 mg/L as initial concentration during the first 24 hours. Such results are in accord with some of the data found by Wong *et al*. (1982) who exposed 3 algal species to short chain trialkyl-tin compounds. The acute toxicity of water treated with MA 1161 towards the organisms entrained through cooling systems seems to of the same order of magnitude as in waters treated with chlorine.

In order to evaluate the actual environmental impact of organo-tin based treatments, the fate of the compound should be considered, including possible loss of biocidal activity during the passage through the plant. Future studies on the persistence of the compound in an active form may be done by utilizing bioassays analysis based on the toxicity curves calculated for *Brachionus plicatilis* and *Artemia salina*, reported with fiducial limit at 95% in Fig. 2. There is evidence that organo-tins can eventually degrade to inert inorganic tin form (Blunden and Chapman 1982). In addition, it has been suggested that the biomethylation of inorganic tin could occur in the environment (Ridley *et al*., 1977).

The amount of Sn that would be discharged by a modern 1200 MW electric power plant utilizing MA 1161 for fouling prevention is approximately 2 tons per year. If such a quantity would be discharged into a river of 1500 m^3/sec flow discharge, the Sn concentration in the water would increase by 60 ng/L.

Such concentration may be considered similar to the highest values reported by Braman and Tompkins (1979), who examined 33 U.S. water bodies, where the max. and min. concentrations they found (as total Sn) were 730 ng/L and 1.3 ng/L respectively with average values of 4.2, 17 and 9.1 ng/L for saline, estuarine and fresh water environments respectively. Although such concentrations of inorganic tin are not reported as toxic for aquatic life, it seems arduous at the present state of knowledge to make any prediction about the possible impact of a continuous discharge of tin into the aquatic environment in comparison with the impact originated by water chlorination treatments.

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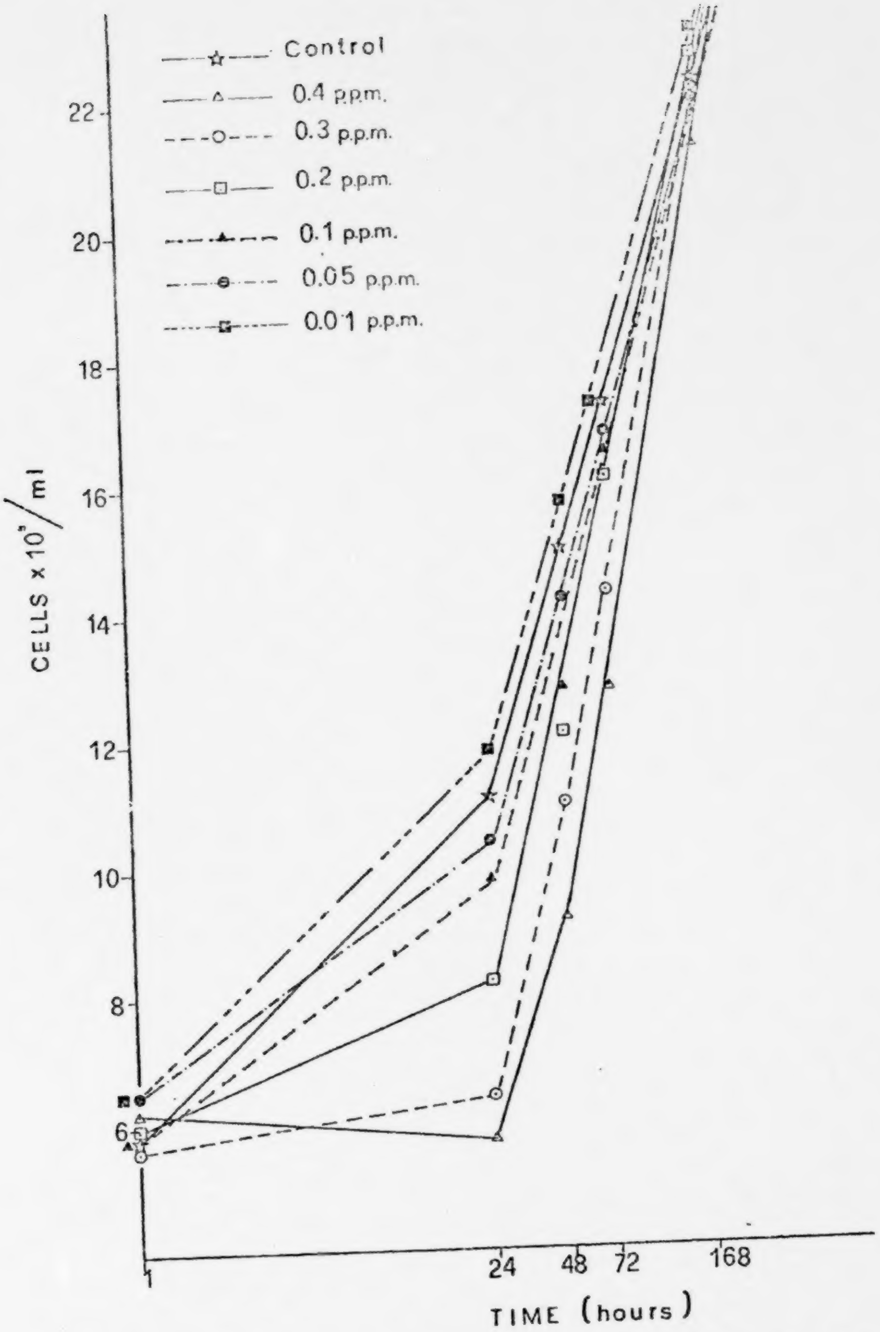

FIG. 1 - EFFECT OF MA1161 CONCENTRATION ON GROWTH OF *Tetraselmis succica*. EACH POINT REPRESENTS THE AVERAGE VALUE OF THREE REPLICATES. THE STANDARD DEVIATION VALUES WERE ± 0.02 - 0.85 (x 10^5) AS MIN. AND MAX. RESPECTIVELY.

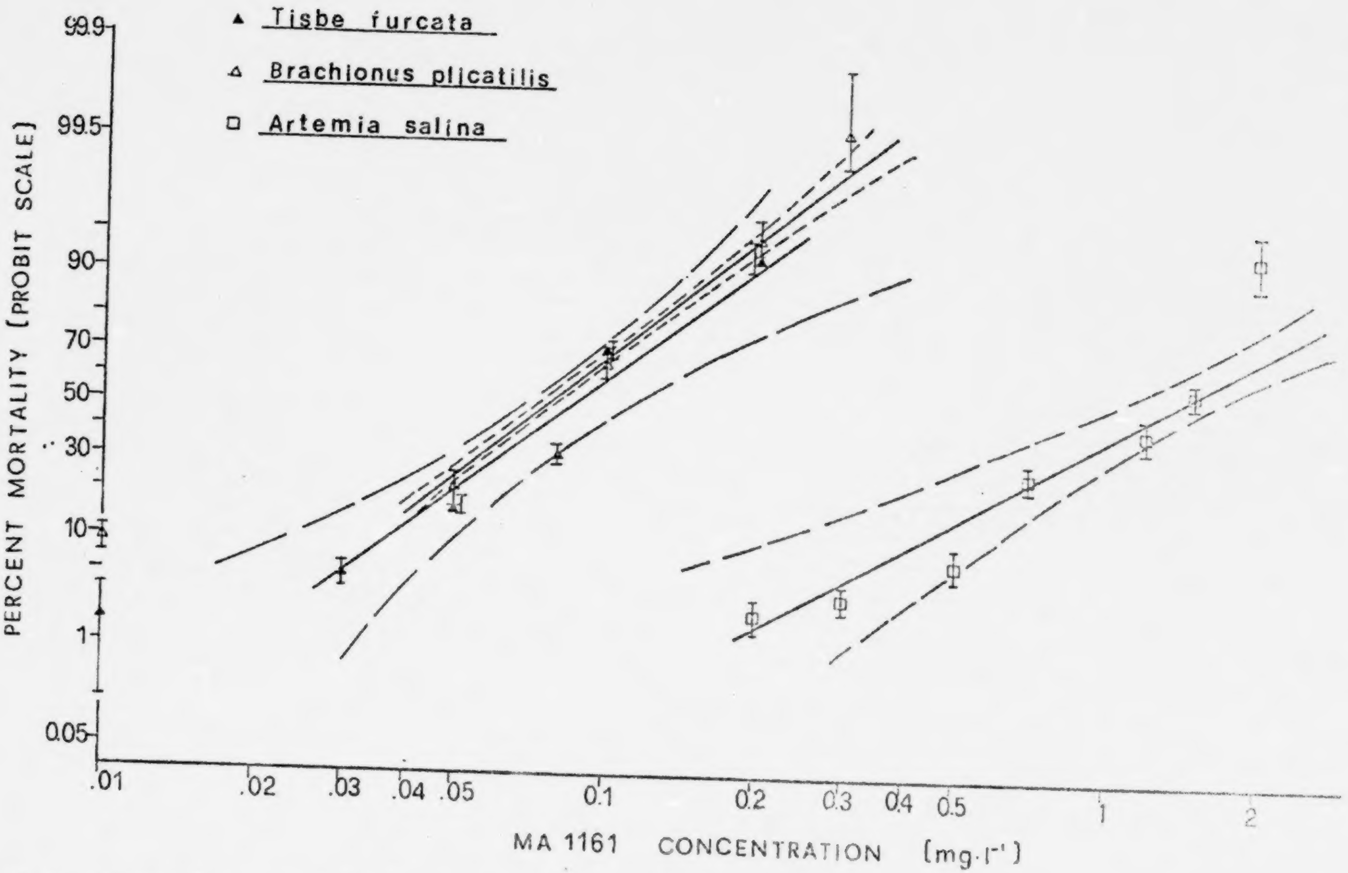

FIG. 2 - TOXICITY OF THE ORGANO-TIN COMPOUND MA 1161 TOWARD THREE INVERTEBRATE SPECIES. EACH POINT REPRESENTS THE AVERAGE VALUE ±S.D. OF THREE REPLICATES WITH 100 ORGANISMS EACH. FIDUCIAL LIMITS AT 95% HAVE BEEN CALCULATED WITH PROBIT ANALYSIS AND ARE INDICATED BY DOTTED LINES. TESTS HAVE BEEN RUN FOR 24 HOURS.

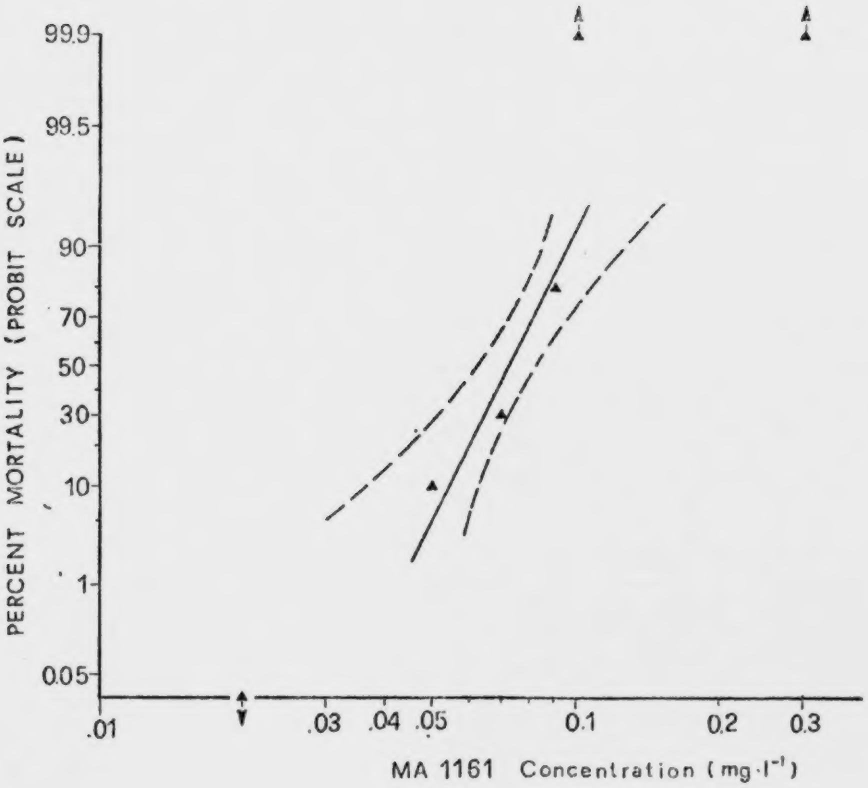

FIG. 3 - TOXICITY OF THE ORGANO-TIN COMPOUND MA1161 TOWARD *Dicentrarchus labrax* AFTER 96 HOURS EXPOSURE. EACH POINT REPRESENTS THE AVERAGE VALUE OF TWO REPLICATES RUN WITH 10 FISH EACH. FIDUCIAL LIMITS AT 95% INDICATED BY DOTTED LINES HAVE BEEN CALCULATED WITH PROBIT ANALYSIS.

VERSATILITY OF ALGAE IN ECOTOXICOLOGY

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SLOTERDIJK, Harm, Pierre COUTURE and Raymond VANCOILLIE. 1982. Versatility of algae in ecotoxicology. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Many ecotoxicological phenomena can be assayed simultaneously using algae as test organisms:

-fertility potential and limiting nutrient factors can be used to show point-source toxicity in watersheds; this has been verified with Selenastrum capricornutum for 4 transboundary (Canada-U.S.) rivers and lakes;

-delayed toxicity of certain substances (Cd, Cu, sylvicor, etc...) can be differentiated from their lethal toxicity when tested with synchronous cultures of Chlorella pyrenoidosa; these tests also allow us to elucidate the mechanisms involved in delayed toxic inductions;

-changes in the toxicity of contaminants and effluents during long-term bioassays can be clearly detected after 1 to 3 days with Chlamydomonas variabilis or Selenastrum capricornutum by measuring their flourescence and/or their assimilation of radiocarbon.

These various applications support the increased utilization of algae as a tool "par excellence" in ecotoxicology.

Key Words: Algae, ecotoxicology, fertility potential, limiting nutrient, Selenastrum capricornutum, delayed toxicity, lethal toxicity, Chlorella pyrenoidosa, Chlamydomonas variabilis.

SLOTERDIJK, Harm, Pierre COUTURE and Raymond VANCOILLIE. 1982. Polyvalence d'utilisation des algues en ecotoxicologie. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les tests avec les algues peuvent déceler plusieurs phénomènes écotoxicologiques en même temps:

-la mise en évidence de toxicités ponctuelles dans des bassins hydrographiques peut être précisée avec leur potentiel de fertilité et l'identification des facteurs limitants; ceci a été vérifié avec Selenastrum capricornutum pour 6 lacs et rivières transfrontaliers Canada/Etats-Unis;

-la toxicité retardée de certains produits (Cd, Cu, sylvicor, etc...) se différencie de leur toxicité létale lorsqu'on les teste avec des cultures synchrones de Chlorella pyrenoïdosa; celles-ci permettent aussi d'expliciter les mécanismes impliqués dans les inductions toxiques retardées;

-les changements de toxicité des contaminants et des effluents lors de longs bioessais se détectent nettement après 1 à 3 jours avec Chlamydomonas variabilis ou Selenastrum capricornutum au niveau de leur fluorescence et/ou de leur assimilation du radiocarbone.

Ces multiples possibilités soutiennent que les algues seront de plus en plus un outil de choix pour les études toxicologiques.

INTRODUCTION

The evaluation of the degree of deterioration of the aquatic environment is increasingly demanding of time and effort. As the effects of pollutants on biological processes in the aquatic environment cannot be adequately assessed from only chemical or physical parameters, several techniques based on the use of biological indicators have been developed. However, toxicity bioassays used for water quality control are restricted too often to provide information on short-term lethal characteristics of the test substance and do not allow the detection of sublethal long-term or delayed toxicities of effluents and natural surface waters.

In this presentation we will discuss three examples of bioassay techniques using algae that evaluated: (i) toxicity in surface water, (ii) toxicity and biochemical parameters, (iii) delayed toxicity.

MATERIALS AND METHODS

Detection of Toxicity in Surface Waters

The toxicity in surface waters can be evaluated by using the fertility potential test, which was developed as the Algal Assay Procedure Bottle Test by the U.S. Environmental Protection Agency EPA (1978). The test was further developed by Chiaudani and Vighi (1978) and by Couture (1981).

The fertility potential and the toxicity factor of river water samples were determined by using a unicellular green alga *Selenastrum capricornutum*.

The present study is described in detail in Couture et al. (1982), so we will only give a brief outline of the methods used.

The water samples were taken from stations in the Tomifobia River, which is located in Southern Quebec near the American border, a mixed forest-agricultural area in the Appalachians. The river is polluted mainly by agricultural run-off. The sampling stations were located from the upstream headwaters area to the mouth of the river in Lake Massawipi. Station locations are indicated in Fig.1.

The samples were prepared and incubated as outlined in Fig.2. The composition of the AAP (Algal Assay Procedure) nutrient medium is listed in Table 1. In order to determine the limiting nutrient factor N or P, the AAP medium was added without N (evaluating whether P is limiting (or without P (evaluating whether N is limiting). The growth stimulating coefficient (GSC) was calculated as outlined in Fig.3.

The concept of the toxicity factor, TF, is based on the ratio of the potential theoretical yield (calculated from chemically determined nutrient concentrations) over the observed experimental yield. This is outlined in Fig.4. The factors 38 and 0.43 were determined by Greene et al. (1975). As can be seen, ratios significantly higher than 1 indicate the presence of toxicity. The addition of EDTA (ethylenediaminetetraacetic acid: 1 mg/L of its sodium salt) to the sample is used to distinguishe between inorganic (metals) toxicity and organic toxicity (Couture, 1981). This chelating agent modifies the availability of inorganic toxic substances by forming a complex and reducing, in certain cases, the toxic effects on algae (EPA, 1978).

Evaluation of Toxicity by Using Biochemical Parameters

The toxicity of various concentrations of copper sulfate and an industrial effluent (source and composition are confidential) have been tested on *S. capricornutum* by measuring Δ-fluorescence, C^{14}-assimilation and increase in cell numbers. The method is described in detail in Van Coillie et al. (1982), and here we will limit ourselves to a brief outline.

S. capricornutum was exposed to various concentrations of the test material for up to 7 days and measurements were made after 1, 4, and 7 days. The culture medium was the regular AAP medium. Cell count was carried out by means of a Coulter Counter. The Δ-fluorescence is based on the increase in fluorescence of the chlorophyll complex after blockage of ATP formation by the herbicide DCMU (3,4 dichlorophenyl - 1,1 - dimethyl urea). This increase depends on the physiological well-being of the algal cells and unfavorable or toxic conditions result in an inhibitory effect (Samuelsson and Oquist, 1977).

In order to determine C^{14}-assimilation, cells were incubated for 10 minutes in the presence of $NaHC^{14}O_3$. After fixing with formaldehyde and releasing excess CO_2 by adding HCl, the chlorophyll was photodegraded. The amount of C^{14} assimilated was estimated by radiocounting using a liquid scintillation counter (Campbell et al., 1976).

Delayed Toxicity

Synchronous cultures of *Chlorella pyrenoidosa* (cellular cyle of 12 hours) were exposed to sublethal concentrations of Sylvicor (dimethyl arsenate, a sylvicide) for 4 hours, starting immediately at the end of a mitosis. Cultures were transferred to a normal AAP medium, after separation by centrifuging. The rate of cellular growth was determined during 4 cycles. RNA synthesis was determined by incubating the culture with radioactive uridine for 60 minutes right after exposure. Subsequent extraction of the RNA allowed the determination of the total RNA fractions spectrophotometrically with orcinol and their radioactivity by measurement in a scintillation counter (Van Coillie et al., 1981).

RESULTS AND DISCUSSION

Detection of toxicity in surface waters

Results are presented in Table 2. The values for the fertility potential indicate that the river is somewhat eutrophic, and markedly so at stations 4 and 18. These stations are downstream from a few small towns which contribute some sewage to the river. Phosphorus was the limiting nutrient at all stations, indicating that eutrophic conditions were not extreme. However, if we compare FP_e (fertility potential-experimental) with total inorganic phosphorus concentrations, we see the high P levels and high FP_e do not correlate. This then indicates that total inorganic P does not necessarily represent the amount of bioavailable phosphorus. Take, for example, st. 20 and 21: the GSC (Growth Stimulating Coefficient) is extremely high, indicating that P was very limiting as a nutrient. However, P's measured (46 and 57 μg/L, respectively) were comparable to many other stations, supporting the idea that varying fractions of total inorganic P are bioavailable.

As we turn to toxicity factors, TF, we see there seems to be toxicity at all stations. However, two stations stand out, st. 20 and 21, where phosphorus was the most limiting factor (GSC → P is 507 and 362, respectively) and where FP_e was the lowest, 0.099 and 0.07, respectively. Often, data like this are interpreted as indicating very toxic conditions, inhibiting algal growth. Indeed, the increase in FP_e by adding EDTA (ratio: 2.8) increases there might be a slight toxicity due to metals. This is however not borne out by the available data on metal concentrations (to be published). In our opinion, the apparent high TF's are more a measure of the lack of bioavailable P than the presence of toxicity. Indeed, we think that the values of TF indicate differences in the bioavailability of P.

Recent findings by White and Payne (1980) and White et al. (1981) confirm that total inorganic phosphorus, as determined by the "Technicon" method without any preanalytical extraction procedure can overestimate the concentration bioavailable to direct uptake by algae. In addition, the acid molybdate used in the Technicon method hydrolyzes part of the organically bound PO_4 to orthophosphate. This overestimation then increases artificially the ratio FP_c/EP_e (calculated fertility potential over experimental fertility potential).

Therefore, it is our opinion that the traditional fertility potential test is not suitable for measuring toxicity, unless other parameters, (for example, those explained in the next section) are used than those outlined above. It does not remain a useful tool for measuring eutrophic conditions and the bioavailability of nutrients.

Evaluation of Toxicity by Using Biochemical Parameters

As was just outlined, toxicity tests using algae, such as S. capricornutum, and the ratio between theoretical and experimental fertility potential have some serious drawbacks. Furthermore, they are lengthy, requiring up to 14 days, and under certain conditions even 21 days. The estimation of biomass using the Coulter counter is subject to error. However, the advantage of using algae is their great sensitivity to certain toxic substances; hence they have been used to evaluate the toxicity of natural surface waters. In our opinion, it is possible to eliminate most of these problems just mentioned by evaluating biochemical parameters and control populations instead of observed cell growth and theoretical cell growth. This is illustrated in the following experiment, which was outlined in the section on Methods and Materials.

Results for copper sulfate are presented in Fig.5. As is to be expected, % I (inhibition) increases with the concentrations of the copper, as is evident in the upper histogram for day 1. Also, cell growth is somewhat less inhibited than are Δ-fluorescence and carbon assimilation. Inhibition is already evident at concentrations as low as 2 μg Cu/L. The IC-50 is around 7 μg Cu/L for day 1.

It is evident from the histogram for day 7 that the solutions with the lower copper concentrations have lost some of their inhibitory effect on cell growth and carbon assimilation, but not their effect on Δ-fluorescence. This decrease is only observed for solutions up to 8 μg Cu/L. The higher concentrations cause a roughly 100% inhibition. The loss of toxicity might possibly be the result of the excretion of polypeptides by the algae, capable of complexing inorganic material and rendering it non-bioavailable. The remaining toxicity in the higher concentrations solutions (16 and 32 μg Cu/L) causes a total inhibition of cell growth, Δ-fluorescence and carbon assimilation after 7 days.

The results for a test with an industrial effluent are presented in Fig.6. There was no toxicity evident at the lower concentrations. Furthermore, there was a significant reduction in toxicity after 4 days. Inhibition has completely disappeared for Δ-fluorescence, but at 90% V/V, cell count showed a 50% inhibition.

These results might indicate that cell count is more sensitive than Δ-fluorescence, contrary to what was observed for $CuSO_4$. However, it was observed that the algal cells showed an agglutination which caused a significant underestimation of the number of cells as determined by a Coulter counter.

Therefore, the greater part of the inhibition is due to cell agglutination and not to true toxicity. In recent studies, Butterwick et al. (1982) and Rehnberg et al. (1982) stated that cell debris can interfere and adversely affect the reliability of cell counts carried out by means of an electronic particle counter.

These results show that cell count is not a very good parameter for evaluating toxicity. Biochemical parameters, such as Δ-fluorescence, are much better measures. Furthermore, toxicity can be detected as early as after 1 day and these short-term bioassays are preferable to long-term bioassays, because, as we saw, toxicity can change significantly after 4-7 days of the test period.

It seems, in our opinion, that toxicity bioassays can easily be carried out using algae, such as Selenastrum capricornutum, and by measuring biochemical parameters of which Δ-fluorescence is the one most easily and quickly done.

Delayed Toxicity

Toxicity bioassays used routinely for water quality control provide information only on lethal characteristics for short duration exposures, and do not allow the detection of delayed toxicities. An experiment to evaluate delayed toxicity was carried out as described in Materials and Methods.

Results of cell growth are presented in Table 3. The control (no sylvicor) had a growth rate approximating the theoretical yield. This indicates that the physical treatment (centrifuging) had very little effect on the growth rate. The exposure to sylvicor diminished the growth rate, the extent of which was dependent on the pesticide concentration. At 12.5 ppb, there was hardly any effect, while at 25 ppb toxic effects only show up 2 cell cycles later. At 50 ppb, toxic effects were apparent immediately.

To elucidate the mechanisms involved in this delayed toxicity, the synthesis of RNA was quantified. Results are presented in Table 4. As can be seen, RNA synthesis is markedly decreased, when cells are exposed to 25 and 50 ppb of sylvicor.

The results of the RNA analysis can explain the delayed toxicity of 2-3 cell cycles, as observed with C. pyrenoidosa treated with 25 and 50 ppb of sylvicor. It has been observed, based on experiments evaluating DNA-RNA transcription in the presence of an inhibitor, actinomycin D, that at the beginning of intermitosis the cell manufactures the RNA precursors necessary for mitoses 2 to 3 cell cycles later (Cameron, 1966). It is therefore logical to assume that sublethal concentrations of sylvicor can induce, by inhibiting the RNA synthesis, a reduction of cell growth 2 to 3 cell cycles later. The mechanisms is briefly outlined in Fig.7.

Thus, algae can be used very easily to determine delayed toxicity at lower levels than at sublethal levels.

CONCLUSION

Toxicity bioassays using the observed and theoretical fertility potential test, the latter based on total inorganic phosphorus, have many drawbacks as was outlined in this paper. The test takes a long time and bioavailable phosphorus concentrations may be overestimated when using the "Technicon" method, especially without any preanalytical extraction procedure.

Therefore, we recommend that other parameters, which are not based on cell growth or increase in cell numbers, be used. In this presentation, the use of biochemical parameters such as Δ-fluorescence and carbon assimilation was discussed, but there are other parameters that could be used (e.g., ATP activity and the adenylate energy charge ratio). Of these various types we recommend tests that can be carried out in a minimum time and are fairly simple, such as Δ-fluorescence. Besides being economic in time and effort, these test are very sensitive and may be applied to the evaluation of surface waters. Most toxicity bioassays are limited to effluents and specific substances, not being sensitive enough to detect toxicity in surface waters.

To complete the concept of ecotoxicology, we must not limit ourselves to lethal tests or to effluents. Sublethal tests evaluating surface waters must be developed. We think that highly sensitive techniques based on algae and biochemical parameters are now available which can be applied to such dilute solutions as surface waters. Algae are organisms "par excellence" for these refined toxicity tests They have proven themselves already to be sensitive to ambient water quality, being so intimately associated with it and possessing a high capacity for extracting and concentrating substances. In future studies, we plan to explore the possibilities of using algae to evaluate surface water toxicity by measuring biochemical parameters such Δ-fluorescence and the adenylate energy charge ratio.

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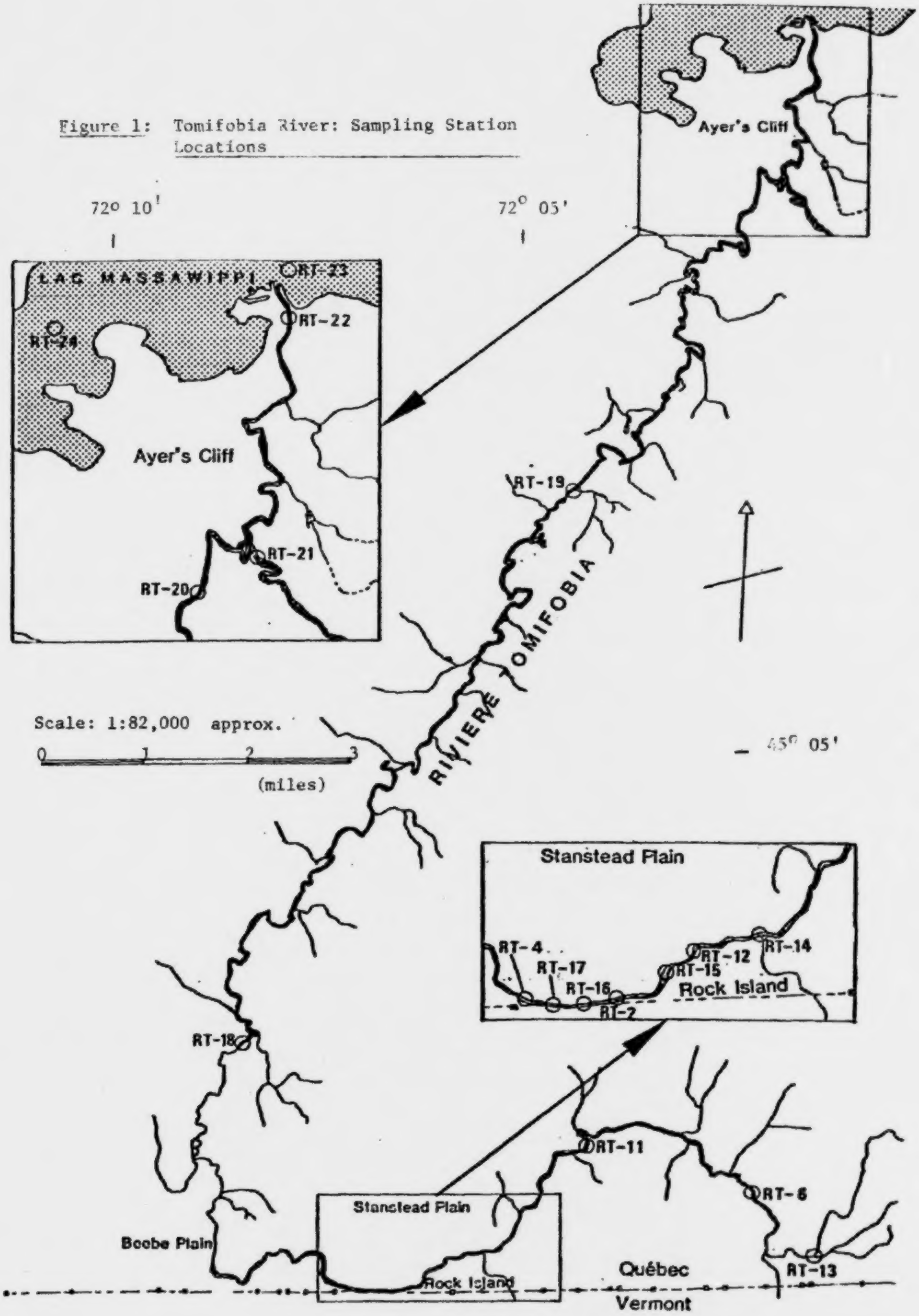


Figure 1: Tomifobia River: Sampling Station Locations

72° 10'
72° 05'
45° 05'
LAC MASSAWIPPI
RT-23
RT-22
RT-24
Ayer's Cliff
RT-21
RT-20
Ayer's Cliff
RT-19
RIVIERE TOMIFOBIA
Scale: 1:82,000 approx.
0
1
2
3
(miles)
Stanstead Plain
RT-4
RT-17
RT-16
RT-2
RT-15
RT-12
RT-14
Rock Island
RT-18
RT-11
RT-6
Stanstead Plain
Beebe Plain
Rock Island
Québec
RT-13
Vermont

Figure 2: Algal Assay Procedure

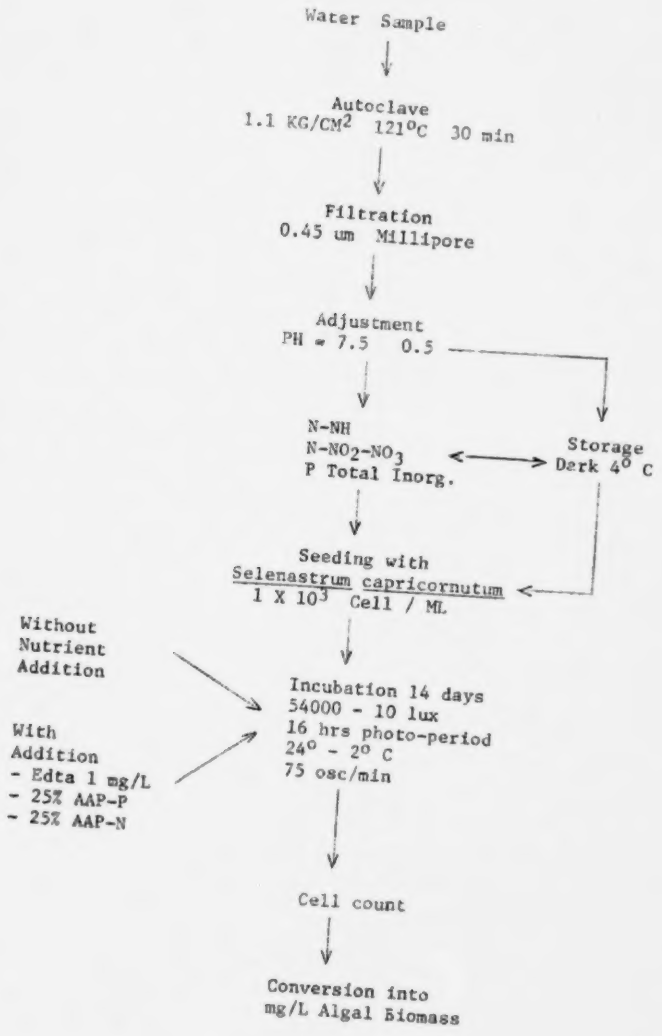

Water Sample
Autoclave
1.1 KG/CM^2 121^{o}C 30 min
Filtration
0.45 um Millipore
Adjustment
PH = 7.5 0.5
N-NH
N-NO$_2$-NO$_3$
P Total Inorg.
Storage
Dark 4^o C
Seeding with
Selenastrum capricornutum
1 X 10^3 Cell / ML
Without
Nutrient
Addition
With
Addition
- Edta 1 mg/L
- 25% AAP-P
- 25% AAP-N
Incubation 14 days
54000 - 10 lux
16 hrs photo-period
24^o - 2^o C
75 osc/min
Cell count
Conversion into
mg/L Algal Biomass

Figure 3: Determination of the limiting nutrient, Nitrogen or Phosphorus

Water Sample – AAP – P → Evaluates N

Water Sample – AAP – N → Evaluates P

$$GSC \rightarrow N^{*} = \frac{FP_{AAP-P}}{FP_e}$$

$$GSC \rightarrow P^{*} = \frac{FP_{AAP-N}}{FP_e}$$

Limiting Nutrient:

N if $GSC \rightarrow N > GSC \rightarrow P$ and ≥ 1.3

P if $GSC \rightarrow P > GSC \rightarrow N$ and ≥ 1.3

* GSC = Growth Stimulating Coefficient.

Figure 4: Calculation of the Toxicity Factor

$$FP_c^{*} = \left[\text{Total } N_I \right] \times 38$$

or

$$FP_c = \left[\text{Total } P_I \right] \times 0.43$$

$$TF = FP_c / FP_e^{**}$$

$$TF \geqslant 1.3 \rightarrow \text{Toxicity}$$

Edta Addition:

IF $$\frac{FP_{e+EDTA}}{FP_e} \geqslant 1.3$$ Then toxicity is probably of an inorganic nature (metals).

* FP_c: Fertility potential as calculated.

** FP_e: Fertility potential as observed in the experiment.

FIGURE 5: Inhibition of S. capricornutum by Copper Sulfate solutions

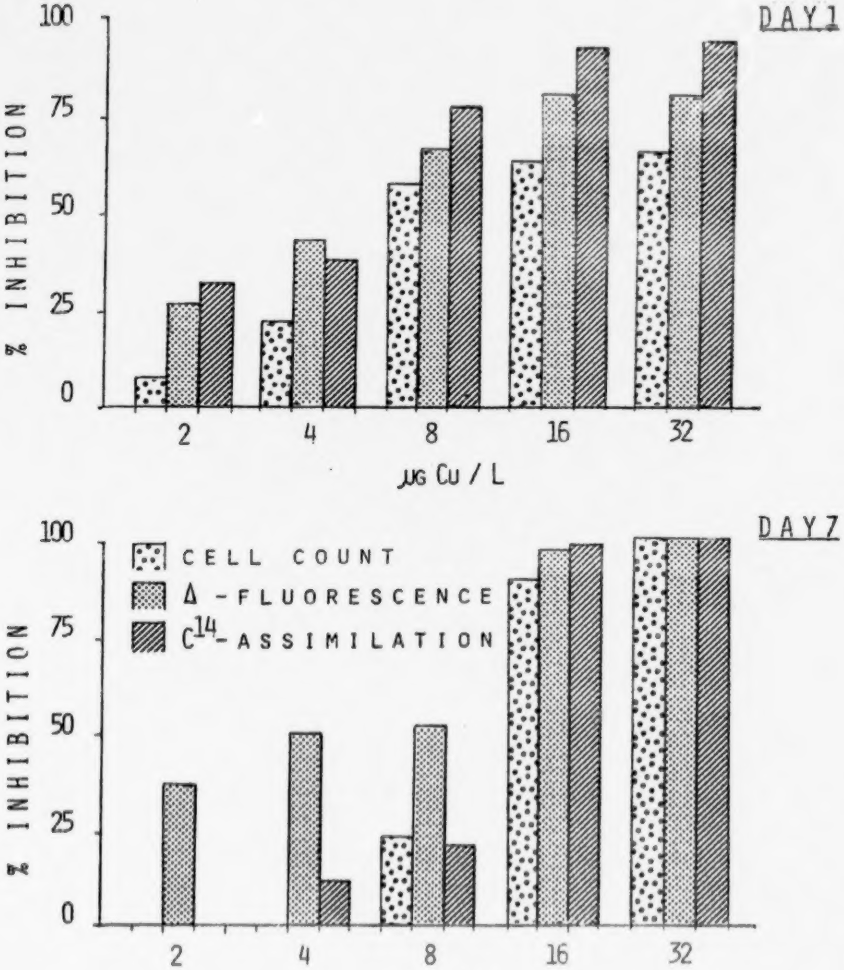
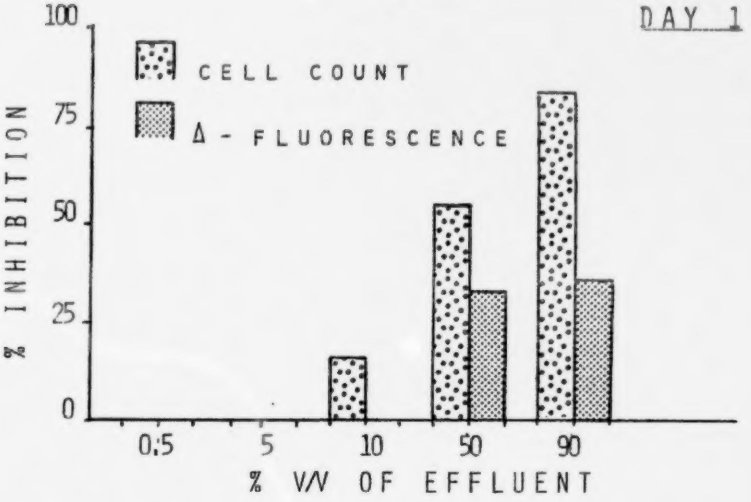


FIGURE 6: Inhibition of *S. capricornutum* by an industrial effluent.



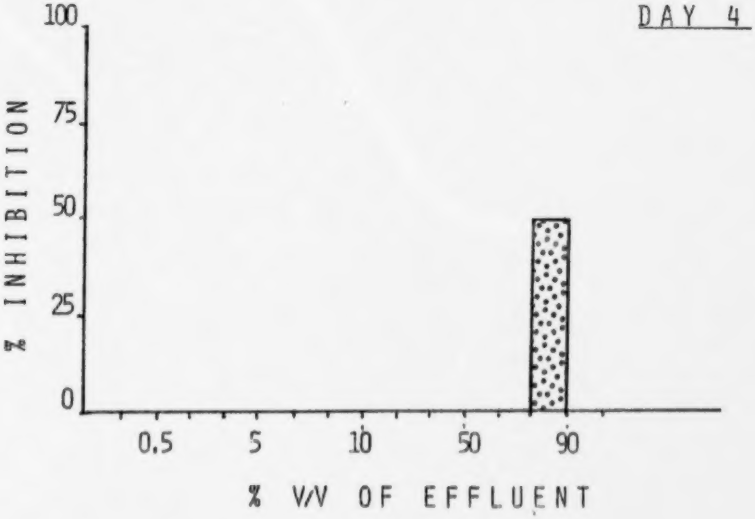


Figure 7: Suggested mechanisme of delayed toxicity

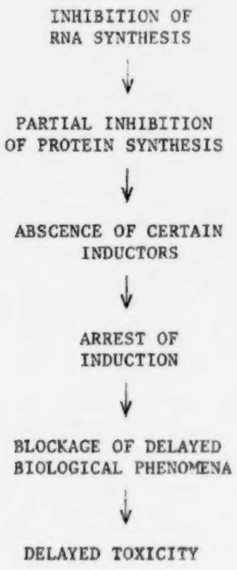

INHIBITION OF RNA SYNTHESIS
PARTIAL INHIBITION OF PROTEIN SYNTHESIS
ABSCENCE OF CERTAIN INDUCTORS
ARREST OF INDUCTION
BLOCKAGE OF DELAYED BIOLOGICAL PHENOMENA
DELAYED TOXICITY

Table 1: Composition of AAP Culture (100%)

ELEMENTS	CONCENTRATIONS
N	4.200 mg/l
P	0.186
C	2.143
K	0.469
S	1.911
Ca	1.202
Mg	2.904
Na	11.001
Mn	115.370 µg/l
Fe	55.000
B	32.460
Mo	2.878
Zn	0.160
Co	0.070
Cu	0.004

Table 2: Toxicity factors and limiting nutrients in the Tomifobia River.

STATION	TF	$\frac{FP_{EDTA}}{FP_e}$ *	$GSC \rightarrow P$ *	FP_e *	TOTAL P_I
RT-13	12	1.1	7.3	1.82	83
RT-6	11	1.1	15.1	1.91	44
RT-11	6	1.1	6.3	4.43	39
RT-14	7	0.9	9.5	3.65	65
RT-12	12	1.1	14.8	2.21	66
RT-15	5	1.2	5.4	5.42	78
RT-2	2	1.5	29.6	1.06	98
RT-16	10	1.2	9.4	3.78	81
RT-17	6	1.0	3.7	6.82	90
RT-4	2	1.0	2.1	14.51	94
RT-18	2	1.0	3.0	12.76	125
RT-19	23	1.5	38.8	1.04	84
RT-20	200	2.8	507.0	0.09	46
RT-22	37	1.4	53.6	0.56	59
RT-21	270	0.7	362.0	0.07	57
RT-23	16	1.4	13.1	1.19	39
RT-24	2	0.9	1.9	9.05	21

* Symbols explained in text and figures 3 and 4

Table 3: Effect of sylvicor on the growth of *Chlorella pyrenoidosa* (Cell count X 10^3/ml)

	0 HR	12 HRS	24 HRS	36 HRS	48 HRS
Theor. Growth	1	8	64	512	1024
Control	1	7.8	63	503	989
12.5 ppb 4 hrs	1	7.8	59	491	957
25 ppb 4 hrs	1	7.5	56	230	318
50 ppb 4 hrs	1	5.3	27	94	173

Table 4: Effect of sylvicor on RNA synthesis in *Chlorella pyrenoidosa*

TREATMENT	RNA SYNTHESIS*		% INHIBITION
Control	95	10	
12.5 ppb 4 hrs	88	11	7
25 ppb 4 hrs	52	8	45
50 ppb 4 hrs	34	4	64

* DPM / μC C^{14} - Uridine incorporated during 60 min/mg RNA in 1 g. dry weight of algae.

EXAMINATION OF EXPOSURE MODELS IN ASSESSMENT OF TOXIC CHEMICALS

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STRACHAN, William. 1982. Examination of exposure models in assessment of toxic chemicals. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Risk/hazard assessment for chemicals involves two essential aspects - exposure and effects (toxicology) - and a comparison of the levels for each. A number of computational models exist for evaluating the exposure of a chemical in the different compartments of a variety of ecosystems. Input for such estimations includes physical-chemical properties and degradation rates. These are used to predict relative concentrations in air, soil, water, sediments, suspended soil, biota (fish) and could be expanded to provide for terrestrial biota as well. The paper discusses several existing models (EXAMS, Mackay FIGACITY',NRC's PERSISTENCE) and compares these input parameters. The need to standardize these parameters before comparing the models is stressed.

Key Words: Exposure models, risk assessment, toxicology, toxic chemicals.

STRACHAN, William. 1982. Examination des modèles d'exposition servant à évaluer les produits toxiques. Can. Tech. Rep. Fish Aquat. Sci. 1163.

L'évaluation des risques et des dangers que présentent les produits chimiques comporte l'étude de deux aspects fondamentaux, soit l'exposition et les effets (toxicologiques), puis la comparaison des concentrations pour chaque cas. Il existe un certain nombre de modèles informatisés servant à évaluer l'exposition des différents compartiment de divers écosystèmes à un produit chimique donné. Pour réaliser ces évaluations, il faut connaître, entre autres, les propriétés physico-chimiques du produit en question et sa vitesse de dégradation. Ces données permettent de prévoir la concentration du produit dans l'air, le sol, l'eau, les sédiments, les solides en suspension et le biote (poisson). Elles peuvent aussi être extrapolées pour le biote terrestre. Le présent document discute plusieurs modèles (EXAMS, FUGACITE´de Mackay, PERSISTENCE du CNR) et on compare entre eux les mêmes paramètres de depart. Il faut souligner la necessité de standardisation de ces paramètres avant la comparsison des modèles.

EXAMINATION OF EXPOSURE MODELS IN ASSESSMENT OF TOXIC CHEMICALS

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INTRODUCTION

Assessment

The introduction of toxic chemicals to the environment from anthropogenic and natural sources is a concern to all persons involved in assessing their impact. The number of commercial chemicals currently stands at approximately 70,000 and grows at some 100-500 per annum. Some of these represent an improvement in the quality of life; others, sometimes even the same ones, represent a potential or real hazard to both man and the other organisms with which he shares the biosphere. An evaluation of this hazard is, or should be, a prerequisite for the establishment of controls.

Hazard and risk seem to have as many meanings as there are participants in the assessment process. Dictionaries indicate that both may be used interchangeably with danger. The one point that most assessors seem to be in agreement on is that with regard to environmental chemicals, they are a function of both exposure and effects (or toxicity). Without exposure of an organism to a toxic substance, there can be no hazard, even if the toxicity is high; conversely, even a gross exposure to a substance does not contitute a hazard if there are no effects.

Exposure

The purpose of this paper is to present and discuss the prediction of the exposure part of hazard evaluation. There are two concepts of exposure possible - actual measurements in the environment and prediction through the use of laboratory determinations of distribution factors. The former is costly and, in a country as large and diverse as Canada, virtually impossible to carry out in a comprehensive fashion. It should be possible to perform such monitoring, however, at locations where chemicals of previously established concern have been identified - and these exposure determinations should be used whenever possible.

The predictive approach is less costly but it is also less reliable than actual measurements. The distribution and subsequent fate of a chemical depends on too many factors for a theoretical estimation to be directly applicable in any particular situation. The models can be made more site-specific, but then they lose their generality. It must be remembered that general statements about a chemical are required and undoubtably will be made; it is up to the scientific community to ensure that they are as reliable as possible.

Uses

Prediction of exposure can be useful in several ways. Actual hazard assessment requires the comparison of concentrations - those for effect levels and those for exposure. Only site-specific models can attempt to provide this. However, it is believed that the use of computerized models providing general exposure estimates can be extremely beneficial to a number of government programs because they are capable of providing an approach to exposure which is logical, consistent and scientifically based. Such models can lead to more consistent and informed decision-making in a cost-effective manner when environmental measurements cannot be provided and yet an evaluation and decision is required.

Models can give estimations of compartmental distribution and hence are very useful in designing surveillance programs. A knowledge of which compartments are important would be of great assistance in selecting media, sites, and sampling frequency. The models can also be used to indicate the sensitivity of the distribution pattern of a chemical to the various input parameters. This would be instructive to researchers who are required to investigate toxic effects on organisms and properties of the chemical(s). Finally, for those who have no option but to make hazard evaluations based on the limited data which may be available at the time, the models provide a consistent basis which can also be rationalized and presented to those whose use of a chemcal may be curtailed in a control action. For this purpose, however, they can only be used in a qualitative manner when comparing exposure and effects.

MODELS

General

The Organization for Economic Co-operation and Development (OECD) has adopted most of the preceding philosophy and approach. A set of Test Guidelines, including a minimum pre-marketing data subset (MPD) has been described (OECD Council, 1981). The MPD is intended to accompany all "new" chemical notifications that many nations are now requiring prior to introduction of a chemical into commerce. The OECD has also described a modeling approach to exposure analysis for hazard evalution (OECD Working Party on Exposure Analysis, 1982). While such modeling is intended for "new" chemicals (for which obviously there will also be no environmental measurements), it is reasonable to believe that this will form the minimum basis for existing chemicals for which such data is also unavailable. Indeed, the OECD is currently moving in this direction with their program on existing chemicals.

Three computerized models were considered for this paper. They are EPA's EXAMS (Lassiter *et al.*, 1979), PERSISTENCE (Roberts *et al.*, 1981), and FUGACITY (Mackay and Paterson, 1981, 1982). All use similar, linearly related, first-order differential equations to describe concentrations and processes as functions of time. They are all capable of considering the impact of transfer and transformation rates on the distribution of chemicals, as well as the influence of emissions to the systems considered. EXAMS and PERSISTENCE restrict themselves to the aquatic environment; FUGACITY makes allowance for the atmospheric and terrestrial compartments as well. EXAMS and PRESISTENCE have the ability to calculate an aquatic photolysis rate constant; FUGACITY zeros this rate in the absence of an experimental value. All programs estimate volatility rates constants.

All three programs allow the user to input a wide variety of equilibrium, rate and system data but PERSISTENCE and FUGACITY will calculate or substitue default values for many of them, given a minimum of other data. In the case of the equilibria constants, calculations in these two programs depend on different empirical relationships; for the rate constants, zero values are inserted except as noted above. Default system parameters are set by the selection of the "scenario" and in their current versions, these differ considerably.

System Properties

It is by definition that concentrations are masses per unit volumes. Consequently, the volumes of the compartments used in each model scenario are critical; so also are the masses but they are seldom available. One approach to avoiding some of the problems in determining concentrations is to calculate relative concentrations instead, using unit mass in the system and volumes proportional to a particular ecosystem. This has the drawback that the 'concentrations' observed cannot be compared directly with effect concentrations in a hazard assessment. It can, however, indicate potentially-impacted media (and hence the organisms which may be at risk) and also the important parameters which influence a chemical's distribution. It may also be possible, through the use of benchmark chemicals, to get a comparative picture of the exposure of a given chemical. Such benchmark chemicals, with known environmental distributions, could be used to rank the exposure of a test chemical or at least to provide strong indications of its likely distribution.

The models all make provision for the input of volumes for each compartment of the particular ecosystem considered. The provision of site specific volumes may add to the relevance in a particular situation and should be used if mass data are also available and if the assessment required is local. For general evaluations, however, standard "scenarios" are recommended as discussed. Those mentioned in this paper are ones related to three aquatic ecosystems - a lake, a pond, and a river. Table 1 indicates the relative default volumes of these scenarios as provided by the three models and also includes the values proposed in the OECD Hazard Assessment Project. For the lake scenario, EXAMS has two versions - oligotrophic and eutrophic. It is apparent that even with the same mass inputs and chemical properties, there will be considerable differences between the predictions. In these scenarios, both versions of EXAMS as well as PERSISTENCE presume less suspended solids than biota in the water column. Particularly when it is recollected that the biota partition coefficient refers to fishes, this seems to be an inappropirate compartment porportion. The volume (or depth) of the 'active' sediment compartment is an unknown. EXAMS uses a depth of 5 cm, PERSISTENCE 1 cm, and FUGACITY, 3 cm. The proportions of the OECD proposal included 10 cm for this compartment, a "guestimate" of a number of experts. The lower levels of the models are also more relevant to the lake scenario than to the pond and river.

The default parameters of the pond scenarios are much more consitent among the models. Only the FUGACITY sediment proportion appears to be substantially different from the other models. It can be argued that a productive pond environment, with little sediment disturbance, will have a deeper active layer than in a lake. PERSISTENCE and FUGACITY have values in keeping with this.

Only EXAMS and FUGACITY make provision for a river scenario but they differ considerably in all compartments. This is due to the level of biota ascribed to the river in EXAMS - 7.2 mg/L. It is suggested that this level is somewhat high when compared with most Canadian situations and the 0.5 mg/L level of FUGACITY seems more reasonable. In this regard, it is worthwhile remembering that biota, in a practical sense, means fish for these models.

Other parameters, besides volumes, are also of importance. Table 2 lists some of these. In the EXAMS lake scenarios, different default parameters are used for biomass and suspended solids in the epilimnion and hypolimnion (the other models assume a homogeneous lake). All of the EXAMS assignments presume equal or greater levels of biota to suspended solids, a situation not in accord with the real world in Canada. It is also suggested that the higher density for the solid matter compartments is more relevant to compacted sediments than to the active layer. The PERSISTENCE model also uses a questionably higher density. In addition, it has two levels of organic carbon in the solid mater - the higher one, for the suspended solids, corresponding to 100% organic matter.

The other parameters in the pond scenarios are all very comparable except for the organic carbon content of the suspended matter. In the river scenarios, EXAMS and FUGACITY differ particularly in the level of suspended solids and biomass. The levels employed by EXAMS (200 mg/L and 7.2 mg/L, respectively) are thought to be unduly high for most Canadian situations.

Based on the preceding considerations, the parameters in Table 3 were selected to be used in comparing the models. The depth or concentrations shown were used, with the areas and densities where appropriate, to calculate the volumes of the four aquatic compartments for each of three aquatic scenarios - lake, pond and river. Other important parameters are also shown. The OECD recommended values are the same as those for the lake except that they are reduced by a factor of 0.3 to allow for land in that model system.

Chemical Properties

The exams model presumes the input (or calculation) of most chemical parameters from outside the program. The PERSISTENCE and FUGACITY models both include empirical equations relating the several partition coefficients to the n-octanol - water coefficient (K_{ow}). Table 4 presents those used along with those recommended in a recent, comprehensive review (Lyman *et al.*, 1982). Mackay (1982) has recently reviewed the relationship for bioconcentration factors and the FUGACITY equation reflects that evaluation. It is interesting to compare these predictions for a selection of compounds for which appropriate data were readily available. Some of the K_{ow}'s employed were the average of a number of determinations while others are for single determinations. For this reason, the comparisons cannot be considered rigorous but hopefully are at least indicative.

There are three compounds for which all solubility predictions (Table 5) differ by at least a factor of 10X from the observed values - di(2-ethylhexyl) phthalate (DEHP), pentachlorophenol and pp'DDT. The deviations are all consistent in direction, however. Excluding these, the relationships in FUGACITY and in Lyman *et al*. appear most suitable. With the exception of anthracene, the PERSISTENCE model predicts consistently high concentrations while the FUGACITY model is somewhat better with ratios closer to unity than those for PERSISTENCE for most determinations. It has, however, two values which differ by a factor of 10 or more from observed values. The equation recommended by Lyman *et al*. for general use, predicts no value differing by 10 or more but the ratios observed are generally greater than the corresponding FUGACITY ones. It is obvious from this that the present state-of-the-art is still only semi-quantitative.

The K_{oc} parameter, together with the fraction of organic carbon content, is used to determine sorption coefficients for sediment and suspended matter. For K_{oc} estimations (Table 6), similar comments to those with solubility can be made. Predictions with the defaults from

PERSISTENCE are universally lower than the observed values. This model also had a high proportion (> 50%) of determinations which exceeded the observed by a factor of at least 10X. The equation recommended by Lyman *et al.* had fewer such extremes than either PERSISTENCE or FUGACITY and appears to be preferable to FUGACITY in its ability to correctly predict K_{oc} since it gives values more randomly distributed about observed values and had fewer values which differed by factors of 10X.

Predictions of bioconcentration factors, BCF (Table 7), using the three default equations, are much better relative to those for solubility or K_{oc}. All models predict all values correctly to within a factor of 5X(FUGACITY fails in this only with pentachlorophenol) and the averages of the experimental:observed values were 1.1, 1.2, and 1.4 for the equations in the PERSISTENCE model, Lyman *et al.*'s book and the FUGACITY model, respectively.

CONCLUSIONS

The preceeding has discussed the input and defaults for some exposure models. It remains to run the models, with identical input data, and to compare the results - amongst the models as well as against environmental data. Preliminary evaluation with fenitrothion, pentachlorophenol and hexachlorobenzene were attempted. Results were ambiguous because of difficulties in overriding some model parameters. Qualitatively, however, there were considerable differences even in defining the compartment with the major fraction of the test substance. There was little consistency in the differences either between the scenario predictions or among the models outputs for any particular scenario. This prelimniary report is being followed up and more extensive evaluation is planned.

ACKNOWLEDGEMENTS

Appreciation is expressed to several persons for running their versions of the models and otherwise providing assistance - Dr. E. Halfon of N.W.R.I. for EXAMS, Prof. D. Mackay of the University of Toronto for FUGACITY and Dr. J.R. Roberts and Ms. M. Mitchell of N.R.C. for PERSISTENCE.

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Table 1: Relative Volumes Of Models ($V_{biota} = 1.0$)

	WATER ($X10^5$)	SEDIMENT ($X10^3$)	SUSP. SOLIDS
LAKE			
a) EXAMS (Olig.)	18	4.7	0.096
b) EXAMS (Eutr.)	6.8	1.8	0.19
c) PERSISTENCE	2.	0.13	0.5
d) FUGACITY	10.	3.	5.
POND			
a) EXAMS	0.77	1.9	1.2
b) PERSISTENCE	1.9	1.9	4.9
c) FUGACITY	2.0	20.	6.6
RIVER			
a) EXAMS	0.15	0.23	1.4
b) FUGACITY	10.	5.	10.
GLOBAL (OECD)	20.	20.	6.6

(plus AIR: 1.7×10^9 and SOIL: 8.6×10^3)

Table 2: Critical Model Parameters

	BIOMASS (mg/L)	SUSP. SOLIDS (mg/L)	ORG. CARBON (%)	DENSITY (mg/L)
LAKE				
a) EXAMS (Olig.)	1.0/0.1	0.1/0.1	0.5	1.85
b) EXAMS (Eutr.)	1.1/0.75	0.5/0.5	3.8	1.85
c) PERSISTENCE	5.5	5.	56/10	4.35
d) FUGACITY	0.5	5.	4.	1.5
POND				
a) EXAMS	12.9	30.	10.	1.85
b) PERSISTENCE	5.0	50.	56/10	2.0
c) FUGACITY	5.0	50.	4.	1.5
RIVER				
a) EXAMS	7.2	200.	1.5	1.85
b) FUGACITY	0.5	10.	4.	1.5
GLOBAL (OECD)	0.5	5.	5.	1.5

Table 3: Standard System Parameters

AREA	LAKE 1Km x 1km	POND 0.1Km x 0.1Km	RIVER 0.01Km x 1Km
WATER:			
Depth (m)	10.	1	3.
Volume (m^3)	1000×10^4	10^4	3×10^4
SEDIMENT:			
Depth (m)	0.1	0.15	0.1
Volume (m^3)	100×10^3	1.5×10^3	10^3
SUSP. SOLIDS*:			
Conc'n (g/m^3)	5.	50.	10.
Volume (m^3)	33.	0.33	0.2
BIOTA (FISH)*:			
Conc'n (g/m^3)	0.5	5.0	0.5
Volume (m^3)	5.	0.05	0.015

* DENSITY - SUSP. SOLID: 1.5 g/ml; FISH: 1.0 g/ml

OTHER PARAMETERS - ORGANIC CARBON: SEDIMENT 4%

: SUSP. SOLID 10%

- RIVER FLOW: 0.1 m/s

Table 4: Default Estimation Methods

	LOG S (mol/L)	LOG K_{oc}	LOG BCF
FUGACITY	$-\mathrm{LOG}\ K_{ow}+0.25$ $-0.010(T_m-20)$	$\mathrm{LOG}\ K_{ow}-0.38$	$\mathrm{LOG}\ K_{ow}-1.32$
PERSISTENCE	$-0.99\ \mathrm{LOG}\ K_{ow}+0.72$ $-0.0095Tm$	$0.56\ \mathrm{LOG}\ K_{ow}-0.12$	$0.85\ \mathrm{LOG}\ K_{ow}-0.7$
Lyman *et al*.	$-1.34\ \mathrm{LOG}\ K_{ow}+0.98$ $-.0097(T_m-20)$	$0.54\ \mathrm{LOG}\ K_{ow}+1.38$	$0.76\ \mathrm{LOG}\ K_{ow}-023$

Table 5: Predicted Solubilities (Relative to Observed)

	LOG K_{ow}	M.P.°C	FUGACITY	PERSISTENCE	LYMAN
NITROBENZENE	1.99	<20	1.1	3.5	1.3
TRICHLOROETHYLENE	2.96	<20	0.2	0.7	0.1
LINDANE	3.62	113	1.5	3.5	0.5
PARATHION	3.81	<20	2.3	4.2	0.4
1,2,4-TRICHLOROBENZENE	4.14	<20	0.9	3.0	0.2
DEHP	4.36	<20	>10	>10	>10
ANTHRACENE	4.50	216	1.5	<.1	0.3
DIELDRIN	4.86	176	>10	>10	1.5
PENTACHLOROPHENOL	5.01	190	<.1	<.1	<.1
HEXACHLOROBENZENE	5.60	231	2.2	6.0	0.2
pp'DDE	5.75	89	>10	>10	1.3
pp'DDT	5.90	109	>10	>10	>10

Table 6. Predicted K_{oc} (Relative to Observed)

	LOG K_{ow}	FUGACITY	PERSISTENCE	LYMAN
TRICHLOROETHYLENE	2.96	3.8	0.3	9.5
LINDANE	3.62	1.9	0.1	2.4
PARATHION	3.81	0.6	<.1	0.6
DEHP	4.36	1.6	<.1	0.9
DIELDRIN	4.86	0.9	<.1	0.3
PENTACHLOROPHENOL	5.01	>10	0.5	>10
HEXACHLOROBENZENE	5.60	>10	0.3	6.4
pp'DDE	5.75	>10	<.1	1.3
pp'DDT	5.90	8.9	<.1	4.1

Table 7. Predicted BDF (Relative to Observed)

	LOG K_{ow}	FUGACITY	PERSISTENCE	LYMAN
NITROBENZENE	1.99	0.3	0.5	1.0
LINDANE	3.62	0.6	0.7	0.7
1,2,3,-TRICHLOROBENZENE	4.14	0.3	0.3	0.4
DEHP	4.36	1.3	1.2	1.4
ANTHRACENE	4.50	1.6	1.3	1.5
DIELDRIN	4.86	0.3	0.2	0.2
PENTACHLOROPHENOL	5.01	6.6	4.7	4.9
HEXACHLOROBENZENE	5.60	1.3	0.8	0.7
pp'DDE	5.75	0.5	0.3	0.3
pp'DDT	5.90	1.3	0.7	0.6

CADMIUM IN AMERICAN LOBSTER (HOMARUS AMERICANUS) FROM THE AREA OF A LEAD SMELTER

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UTHE, J.F., C.L. CHOU, D.G. ROBINSON and R. Levaque CHARRON. 1982. Cadmium in American lobster (Homarus americanus) from the area of a lead smelter. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Extremely high cadmium levels were found in hepatopancreas of lobster captured in Belledune Harbour situated in northeastern New Brunswick. Claw and tail meat levels of cadmium, while much lower, were high enough to warrant closure of the harbour to commercial fishing. Lobster wandering complicated study of the situtation. Tagging-recapture studies showed that 20.5% of the animals which had been tagged in the harbour wandered out of the harbour, yet highly contaminated animals were rarely found outside of the harbour. A tight relationship was observed between the total amount of cadmium in the hepatopancreas and that in the cooked tail and claw meat. Controls installed in the smelter in 1980 have resulted in decreased levels of cadmium in lobsters captured in the spring of 1982.

Key Words: American lobster, Homarus americanus, lead smelter, cadmium, hepatopancreas, tail meat, tagging-recapture, claw meat.

UTHE, J.F., C.L. CHOU, D.G. ROBINSON and R. Levaque CHARRON. 1982, Présence de cadmium chez le homard américan (Homarus americanus) vivant à proximité d une fonderie de plomb. Can. Tech. Rep. Fish Aquat. Sci. 1163.

En 1980, des niveaux extrêmement élevés de cadmium ont été trouvés dans l'hepatopancréas de homards capturés dans le port de Belledune, situé au nord-est du Nouveau-Bruncwick. Bien que les niveaux de cadmium trouvés dans la chair de la queue et des pinces aient été beaucoup moins élevés, il n'en a pas moins été nécessaire d'interdire la pêche commerciale dans le port. L'errance des homards a compliqué l'étude de la situation. Cependant, des études de capture-recapture ont démontré qu au moins 20.5% des homards marqués dans le port, en sont sortis. Toutefois, des homards fortement contiminés ont rarement été retrouvés à l'extérieur du port. Une forte corrélation a été observée entre la concentration totale de cadmium dans l'hépatopancreas et celle de la chair cuite de la queue et des pinces. Des mesures de contrôle mises en place à la fonderie de plomb en 1980 ont contribué a une diminution des niveaux de cadmium dans les homards capturés au printemps de 1982.

Cadmium in American lobster (Homarus americanus) from the area of a lead smelter

by

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Since 1980 the Department of Fisheries and Oceans with the cooperation of Brunswick Mining and Smelting Corporation Ltd. has been investigating cadmium (Cd) levels in lobsters (Homarus americanus) from the area of Belledune Harbour, New Brunswick, Canada. Details of the studies carried out during 1980 and 1981 can be found in Uthe and Zitko (1980) and Uthe et al. (1982). This report will document the results of studies carried out during 1982. All of the methods and materials used in 1982 are described in the above reports.

Due to the presence of high cadmium levels in the hepatopancreas of lobsters from Belledune Harbour, the harbour was closed to commercial fishing in 1980 and has remained closed since then. In addition, all lobsters taken in a zone surrounding the harbour were processed following removal and discarding of the carapace section, including the hepatopancreas from the live animal. Only the meat from the tail and the claws was used to prepare products. Product inspection ensured that no contaminated meat reached the market. In November 1981 a major effluent treatment facility was opened at the smelter. This and other control measures have decreased the cadmium discharges by more than 95%.

Table 1 shows that the mean level of hepatopancreatic cadmium in lobsters captured at various locations within and around Belledune Harbour appears to have peaked in 1981 (Table 1) although significant decreases have occurred only at the sites labelled L4E and L6E, both located outside and downstream of the harbour. As in previous years, the geographical distribution is characterized by extremely high cadmium levels in harbour animals, markedly lower levels in lobsters captured immediately outside the harbour and a gradually decreasing cadmium mean level as the downstream distance from the harbour increases. The site immediately upstream of the harbour (L1W) is similar in level to the L6E site in spite of being much closer to the harbour mouth.

Table 2 show the levels of cadmium in cooked meat (tail and claw meat pooled) of lobsters captured at the same sites as in Table 1 and the corresponding cadmium levels in the uncooked hepatopancreas from the same animals. It should be noted that the geometric mean cadmium levels in the hepatopancreas differ from those in Table 1 because of the different numbers of animals used in each sample. In general, the level of cadmium in the cooked meat averaged 150 to 400 times lower than the corresponding hepatopancreatic levels. The cooked meat cadmium mean levels were generally lower in 1982 than 1981, but it should be noted that the mean levels of cadmium in the hepatopancreas were also somewhat lower in 1982 with the exception of the LIE sample. There was, however, no statistically significant decrease in cooked meat cadmium levels between 1981 and 1982 by analysis of covariance with weight as the covariant. This is likely an effect of the small sample size and the limited ability of the statistical analysis to accurately correct for the effect of the differences in the total weights of the animals making up the sample (Uthe et al. 1982).

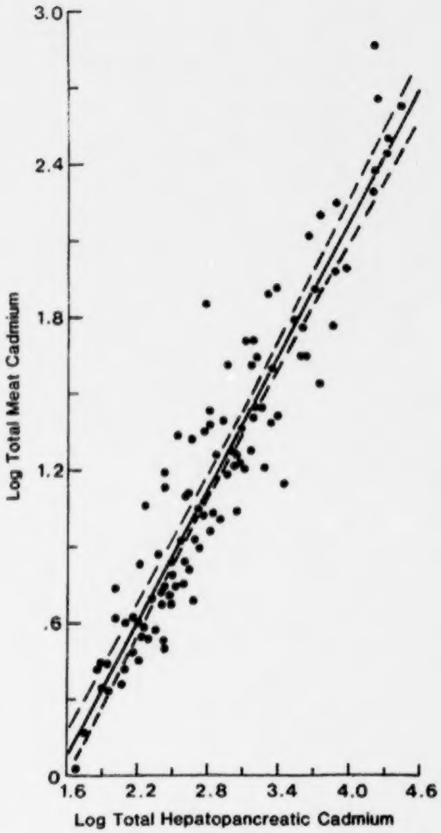

Figure 1 - Log-log relationship between the total amount (μg) of cadmium in cooked, pooled lobster tail and claw meat and that in the hepatopancreas of the same animal. (Solid line + 95% confidence limits.)

In collaboration with the Inspection Service, Gulf Region, Department of Fisheries and Oceans, a pilot operation to prepare lobster products from various mixtures of harbour and controlled fishery zone lobsters was carried out in 1982. The results of this study are shown in Table 3. While there is a trend of increasing cadmium levels in products prepared with increasing amounts of harbour animals none of the mean levels were significantly different from each other. The mean cadmium levels in commercial products prepared from the controlled

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fishery zone during the commercial fishing season in 1981 and 1982 were 0.20 and 0.19 μg/g respectively with a range of 0.07 - 0.30 μg Cd/g in 1981 and 0.04 - 1.25 μg Cd/g in 1982. The overall mean cadmium level in products prepared with equal amounts of harbour and controlled fishery zone lobsters was 0.28 μg/g. This level is very close to the level deemed fit for human consumption for the products from the controlled fishery zone (approximately 0.29 μg/g). The lack of a significant difference in cadmium levels in products prepared solely from harbour animals means that the reopening of the harbour to commercial fishing as a part of the current controlled fishery zone would not entail any change in the current fishery operations which ensures special processing and inspection of the resulting product. It is estimated that fishing the harbour would yield about the same weight of lobster as from the current controlled fishery zone (28,000 lbs.). Thus, on average, products prepared from animals from these two areas would have about 50% meat from harbour animals and about 50% meat from animals from the controlled fishery zone.

The relationship between the total quantity (μg) of cadmium present in the cooked meat and the total amount of cadmium present in the uncooked hepatopancreas is shown in Figure 1 for all the sample sites combined (1982 data). The relationship is highly significant with a correlation coefficient of 0.939 and is described by the equation:

$$\mathrm{Log}_{10}\mathrm{Cd}_{\text{cooked meat}} = -1.321 + 0.876\ \mathrm{Log}_{10}\mathrm{Cd}_{\text{raw hepatopancreas}}$$

Acknowledgements

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Uthe, J.F. and V. Zitko (Editors). 1980. Cadmium pollution of Belledune Harbour, New Brunswick, Canada. Can. Tech. Rep. Fish. Aquat. Sci. 963. v + 107p.

Table 1 - Geometric mean cadmium levels (μg/g wet weight) in hepatopancreas of lobster captured within or around Belledune Harbour, New Brunswick.

Distance from harbour	Sample site	1980			1981			1982	
		$\overline{X}_g$	No. of animals	ANOVA 1980/81	$\overline{X}_g$	No. of animals	ANOVA 1981/82	$\overline{X}_g$	No. of animals
30 km West	Heron Island	3.85	30	S.[a]	4.83	27	N.S.	4.69	23
1.6 km West	LIW	11.9	29	N.S.	20.	28	N.S.	14.3	33
Within Belledune Harbour	Harbour West	176.	29	S.	210.	46	N.S.	147.	35
	Harbour East	62.3	28	N.S.	68.5	44	N.S.	76.3	35
Outside Harbour Breakwater	LOBE	21.4	29	S.	65.7	27	N.S.	46.7	27
3.1 km East	LIE	28.1	31	S.	40.6	34	N.S.	40.4	36
7.8 km East	L4E	28.0	26	S.	42.7	48	S.	23.8	38
10.0 km East	L6E	17.3	19	S.	25.5	31	S.	15.6	36
	Limestone Point[b]	-	-	-	13.6	26	N.S.	13.9	32
17.4 km East	Petit Rocher	11.6	31	N.S.	15.7	41	N.S.	12.9	36

a. $p < 0.05$
b. not sampled in 1980

Table 2 - Mean and range of cadmium levels (µg/g wet weight) in cooked meat pools and raw hepatopancreas lobster from the area of Belledune Harbour, New Brunswick (1982)

		Cooked meat		Raw hepatopancreas		
Sample site	Year	Xg	Range	Xg	Range	N
Heron Island	1981	0.04	0.03-0.09	5.80	4.36- 12.0	10
	1982	0.03	0.02-0.03	5.32	3.51- 10.1	9
L1W	1981	0.12	0.03-1.82	29.6	8.30-135.	10
	1982	0.03	0.03-0.06	8.77	5.76- 17.5	6
Harbour West	1981	0.89	0.16-3.06	218.	62.7 -572.	8
	1982	0.74	0.11-3.26	165.	33.3 -728.	21
Harbour East	1981	0.35	0.07-1.28	67.9	17.0 -355.	11
	1982	0.23	0.04-0.762	62.5	14.7 -470.	12
LOBE	1981	0.28	0.05-1.24	79.6	13.5 -308.	10
	1982	0.16	0.02-1.14	41.0	6.7 -190.	13
LIE	1981	0.21	0.05-0.68	34.3	8.15-130.	10
	1982	0.18	0.04-0.33	47.8	10.3 -134.	12
L4E	1981	0.19	0.05-0.43	54.6	7.67-127.	10
	1982	0.11	0.03-0.20	23.4	11.5 - 47.	11
L6E	1981	0.11	0.05-0.28	30.7	12.2 - 50.9	10
	1982	0.08	0.02-0.14	19.5	5.8 - 54.3	8
Beach Point, P.E.I.	1981	0.03	0.02-0.09	11.8	4.3 - 49.9	10
Petit Rocher N.B.	1982	0.06	0.04-0.10	14.0	7.1 - 32.3	10

Table 3 - Cadmium levels (µg/g wet weight) in frozen commercial lobster packs (320g) prepared from lobsters captured within Belledune Harbour (Harbour West (HW) or Harbour East (HE) sites) and the controlled fishery zone (CFZ) (1982).

Composition	N	$\overline{X}$ ± S.D.[a]	Range
25% HW 25% HE 50% CFZ	6	0.279 ± 0.055	0.238-0.380
50% HW 50% CFZ	6	0.237 ± 0.117	0. 06-0.382
50% HE 50% CFZ	6	0.310 ± 0.184	0.202-0.542
Mean of above 3		0. 28	
100% HW	6	0.313 ± 0.112	0.183-0.480
100% HE	6	0.317 ± 0.209	0.133-0.726
Mean of above 2		0. 32	
100% CFZ	6	0.164 ± 0.118	0.084-0.354

a. No significant differences at $p = 0.05$

QUANTITATIVE STRUCTURE-ACTIVITY RELATIONS OF LETHAL AND SUBLETHAL EFFECTS OF BENZENE AND PHENOL DERIVATIVES ON RAINBOW TROUT

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KAISER, K.L.E., P.V. HODSON and D.G. DIXON. 1982. Quantitative structure-activity relations of lethal and sublethal effects of benzene and phenol derivatives on rainbow trout. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

From the limited data available so far, the following QSAR equations were derived:

a) for phenols:
- log (conc. oral LD50) = 1.35 log P + 0.07 MR - 2.85 (1)
- log (conc. 50% SSDH increase) = 0.93 log P - 1.05 (2)

b) for benzenes:
- log (conc. IP LD50) = 0.21 log P - 1.79 (3)

where concentrations are expressed in μmole/L, P is the n-octanol/water partition coefficient and MR is the molar refraction. However, some symmetrical chlorobenzenes (B); i.e., 1,3,5-Cl_3-B, 1,2,4,5-Cl_4B, and probably Cl_6-B do not appear to follow equa. 3; their toxicities are significantly lower than those predicted from this equation.

Key Words: Partition coefficient, chlorobenzenes, rainbow trout, Salmo gairdneri, benzene derivatives, phenol derivatives.

KAISER, K.L.E., P.V. HODSON and D.G. DIXON. 1982. Relations quantitatives structure-activité des effets létaux et sublétaux des dérivés du benzène et du phénol sur la truite arc-en-ciel. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On a établi des corrélations quantitatives structure-activité pour les dérivés du phénol et du benzène. Voici les effets étudiés: dose létale médiane déterminée 96 heures après l'administration d'une dose unique par voie orale ou par voie intrapéritonéale, dans une matrice d'eau ou d'huile de foie de morue, concentrations létales médianes lors dosage biologique en continue de 96 heures; et seuil de réponse de l'activité de la sorbitol-déshydrogénase sérique 48 heures après injection intrapéritonéale. Parmi les substances chimiques étudiés, on compte le phénol non-substitué et les phénols para-substitués suivants: méthoxy-(CH_3O), phénoxy-(C_6H_5O), chloro-(Cl), méthyl-(CH_3), hydroxy-méthyl-(CH_2OH), ion méthyl-ammonium-(NH_2-CH_3^+), nitro-(NO_2), trifluorométhyl-(CF_3), cyano-(CN) et amino-(NH_2). On a également étudié le benzène (B) et les dérivés chlorés et fluorés que voici: Cl-B, 1,2-Cl_2-B, 1,3-Cl_2-B, 1,4-Cl_2-B, 1,2.3,-Cl_3-B, 1,2,4-Cl_3-B, 1,3,5-Cl_3-B, 1,2,3,4-Cl_4-B, 1,2,3,5-Cl_4-B, Cl -B, Cl_6-B, et F_6-B.

On a établi par analyse de régression par la méthode des moindres carrés, une corrélation entre les effets aigus et sublétaux, exprimés comme l'inverse du logarithme des concentrations en mmole.L^{-1} ou en mmole.kg^{-1} (de masse corporelle) respectivement, et les propriétés moléculaires suivants: coefficient de partage octanol/eau (log P), réfractivité molaire (RM), constantes de champ (F) et constantes de résonance (R) de Swain et de Lupton. Les valeurs mesurées et calculées de la toxicité sont données aux Tableaux 1 et 3. Les équations de régression et les paramètres statistiques sont donnés au Tableau 2.

Extended Abstract

Substituted phenols and benzenes are widely used chemicals which have been found to contaminate aquatic systems. For example, chlorophenols have been observed in several of the Great Lakes (Jones, 1981), and chlorobenzenes are common contaminants of fish tissues from both freshwater and marine habitats.

As part of our ongoing studies on quantitative structure-activity correlations (QSAR) of environmental contaminants, we report here the lethal and sublethal effects of selected phenol and benzene derivatives on juvenile rainbow trout (*Salmo gairdneri*) and empirical correlations with physical parameters allowing quantitative predictions of the observed effects.

The acute lethal effects tested were the medial lethal doses determined 96 hr after administration of a single oral dose (OLD50) or by intraperitoneal injection (IPLD50) in an aqueous or cod liver oil matrix, and the median lethal concentrations in 96-hr flow-through bioassays (LC50). The sublethal effect was the response threshold for serum sorbitol dehydrogenase activity 48 hr after intraperitoneal injection (SSDH). The threshold levels were determined from the intercept of the upper 95 percent confidence limit of the control values with the slope of the dose-response curve on a double logarithmic plot. The experimental details of the toxicity tests have been described elsewhere (Dixon *et al.*, 1982, 1983).

All chemicals used were of the highest purity commercially available and were further purified by fractionation or recrystallization. Concentrations of the chemicals for the aqueous exposure tests were measured daily in each tank, and LC 50 values were calculated from measured exposure concentrations. All toxicity data are expressed in the logarithmic form, $\log\frac{1}{c}$, where c is the concentration or dose, given in $mmole \cdot L^{-1}$ or $mmole \cdot kg^{-1}$ (body weight), respectively.

For the ASAR equations, several common bulk and/or molecular properties were examined for their correlations with the observed effects. In particular, octanol/water partition coefficients (log P), molar refractivity (MR), and Swain and Lupton's field (F) and resonance (R) constants as given by Hansch and Leo (1979) were used.

Table 1 gives four different toxicity or effect data on rainbow trout for a set of para-substituted phenols. Each effect column, IPLD50, OLD50, SSDH threshold, and LC50, lists two sets of data; at the left the measured, and at the right, calculated values. The calculated values were those obtained from the equations given in Table 2. These equations were determined from least square multiple regression analysis. Compounds for which no calculated values are given in Table 1 were excluded from the regression equation; they are phenol in the IPLD50, and para-methylammonium ion in the OLD50 and LC50 tests. The compounds listed in Table 1 are phenol (-), and the following para-substituted phenol derivatives: methoxy - (CH_3), phenoxy - (C_6H_5O), chloro - (Cl), methyl- (CH_3), hydroxymethyl - (CH_2OH), methylammonium ion - (NH_2-CH_3^+), nitro - (NO_2), trifluoromethyl- (CF_3), cyano - (CN), and amino - (NH_2).

Table 1. Measured and calculated effect levels of a variety of para-substituted phenols on acute and sublethal toxicity to rainbow trout. Correlation equations are given in Table 2. OLD50: median lethal oral dose; IPLD50: median lethal intraperitoneal injection dose; SSDH: median response threshold for serum sorbitol dehydrogenase activity; LC50: median lethal concentration in 96-hr flow-through bioassay.

Position/ Substituent	IPLD50		OLD50		SSDH		LC50	
	$\log\frac{1}{c}$		$\log\frac{1}{c}$		$\log\frac{1}{c}$		$\log\frac{1}{c}$	
	meas.	calcul.	meas.	calcul.	meas.	calcul.	meas.	calcul.
-	-0.65	-	-0.75	-0.76	-2.23	-2.04	0.99	0.79
4-CH_3O	-0.46	-0.48	-0.52	-0.45			0.64	0.75
4-C_6H_5O	0.03	0.01			-2.58	-2.49		
4-Cl	0.04	0.05			-1.64	-1.78	1.83	1.85
4-CH_3	0.15	0.08	0.06	0.15	-2.18	-2.35	1.16	1.18
4-CH_2OH	0.16	0.30						
4-$NH_2CH_3^+$	0.25	0.34	0.14	-			2.62	-
4-NO_2	0.52	0.55	0.34	0.23	-1.13(?)	-1.10	1.24	1.15
4-CF_3	0.56	0.57						
4-CN	0.74	0.58					0.72	0.86
4-NH_2							1.96	1.96

In addition to the phenol data (Table 1), a set of IPLD50 data of halogenated benzenes was obtained (Table 3). The regression equation for these compounds is given in Table 2. The series contains benzene, chlorobenzene, pentachloro- and hexafluorobenzene, three dichloro-, two trichloro- and two tetra-chloro-, pentachloro-,and hexafluorobenzene. Three of the compounds tested, namely 1,3,5, trichloro-, 1,2,4,5,-tetrachloro- and hexachlorobenzene, had measured IPLD50 values much below those calculated from this QSAR equation and were excluded from the calculation. It is of interest to note that these three compounds have a high degree of molecular symmetry.

Table 2. Number of measured toxicity values (n), correlation coefficients (r^2), standard deviations (s.d.), coefficients and parameters of correlation equations of toxicity data shown in Tables 1 and 3.

Compounds	Effect$^{(a)}$	n	r^2	s.d.	Equation$^{(b)}$
Phenols	OLD50	4	0.97	0.08	$\log\frac{1}{c} = -2.85 + 1.35 \log P + 0.070\ MR$
Phenols	IPLD50	9	0.94	0.09	$\log\frac{1}{c} = 0.21 + 0.012\ MR + 1.54\ R$
Phenols	SSDH	5	0.93	0.15	$\log\frac{1}{c} = -1.29 - 0.51 \log P + 1.73\ F$
Phenols	LC50	7	0.95	0.12	$\log\frac{1}{c} = 2.04 - 2.15 \log P + 0.88 (\log P)^2$
Benzenes	IPLD50	11	0.78	0.09	$\log\frac{1}{c} = -1.71 + 0.20 \log P$

(a) See Table 1 for abbreviations.
(b) P: Octanol/water partition coefficient; MR: molar refractivity; R: resonance constant: F: field constant.

Table 3. Measured and calculated effect levels of chloro- and fluoro-substituted benzene derivatives to rainbow trout. The correlation equation is given in Table 2.

Position/Substituent	IPLD50		SSDH	
	$\log\frac{1}{c}$ meas.	$\log\frac{1}{c}$ calcul.	$\log\frac{1}{c}$ meas.	$\log\frac{1}{c}$ calcul.$^{(a)}$
-	-1.41	-1.28	-0.72	
Cl	-0.98	-1.14	-0.15	
1.2-Cl_2	-0.87	-1.00		
1.3-Cl_2	-1.00	-0.99		
1.4-Cl_2	-1.01	-0.98	-0.42	
1.2.3-Cl_3	-0.95	-0.89		
1.2.4-Cl_3	-0.99	-0.92	-0.59	
1.3.5-Cl_3	-1.48	-		
1.2.3.4-Cl_4	-0.69	-0.81	-1.84	
1.2.3.5-Cl_4	-0.89	-0.81		
1.2.4.5-Cl_4	-1.37	-		
Cl_5	-0.76	-0.73	-1.25	
Cl_6	-1.24	-		
F_6	-1.27	-1.27		

(a) Analysis in progress.

Our results show the existence of structure-activity correlations of certain chemical and physical parameters with the observed biological effects of various phenol and benzene derivatives on rainbow trout. However, some of the measured values were excluded from the QSAR equations and some of the data sets are extremely small. Therefore, any extrapolation to other compounds may not be warranted until more data are collected.

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BIOASSAY DETERMINATIONS OF FREE METAL ION CONCENTRATIONS TOXIC TO AQUATIC BIOTA

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BORGMANN, U. and K.M. RALPH. 1982. Bioassay determinations of free metal ion concentrations toxic to aquatic biota. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Many researchers feel it is important to relate metal toxicity to the concentration of the free metal ion. However, limitations in the sensitivity of chemical methods for measuring free metal concentrations can make this difficult. Consequently, we have developed and tested a bioassay technique for determining free metal concentrations. This involves determination of metal toxicity before and after addition of a weak complexing agent (i.e., metal ion buffer) with known complexing ability. Tests with Daphnia in defined media demonstrate that the assay can determine free copper concentrations even in the presence of toxic metal complexes.

Key Words: Free metal ion, bioassay, Daphnia, copper, metal complexes.

BORGMANN, U. and K.M. RALPH. 1982. Détermination à l'aide d'épreuves biologiques des concentrations d'ions métalliques libres toxiques pour le aquatique. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Selon de nombreux chercheurs, il est important d'établir une relation entre la toxicité d'un métal et la concentration de ses ions libres. Toutefois, la faible sensibilité des méthodes utilisées pour doser les espèces métalliques libres rend cette tâche difficile. Nous avons donc mis au point et éprouvé une méthode permettant de déterminer la concentration d'espèces métalliques libres. Cette méthode comporte la mesure, à l'aide d'épreuves biologiques, de la toxicité avant et après l'addition d'un complexant faible (c.-à-d. un tampon de l'ion métallique) dont on connaît la capacité de chélation. Des essais effectués avec Daphnia dans un milieu bien défini montrent qu'on peut ainsi déterminer la concentration des espèces libres du cuivre même en présence de complexes de métaux toxiques.

THE EFFECTS OF LOW pH AND CALCIUM ON THE RELATIVE BIOACCUMULATION OF Se-75 and Hg-203 BY SEVEN TISSUES OF THE CRAYFISH, *ORCONECTES VIRILIS*

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CHANG, P.S.S., D.F. MALLEY and J.F. KLAVERKAMP. 1982. The effects of low pH and calcium on the relative bioaccumulation of Se-75 and Hg-203 by seven tissues of the crayfish, *Orconectes virilis*. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Contamination of aquatic systems by Se and Hg is increasing in association with the phenomenon of acidic precipitation. Accumulation of Se-75 and Hg-203 was measured in seven tissues of crayfish held in cages in 1-m diameter enclosures in Lake 302, Experimental Lakes Area, northwestern Ontario. Data for Hg-203 are reported elsewhere. Se-75 was accumulated in tissue (cpm.g^{-1}) in the following order: hepatopancreas>green gland>gut>ovary>gills>carapace>abdominal muscle: In most cases, crayfish accumulated Se-75 to a greater extent at pH 5.4 than at 6.7 Se-75 accumulation was enhanced by the addition of Ca^{++} under acidic conditions. Acidification had no effect on the Se-75:Hg-203 ratios in tissues, but crayfish exposed to pH 5.4 and 5.0 with enriched Ca^{++} (5, 15 mg.L^{-1}) showed significantly higher ratios than those under neutral conditions with or without added Ca^{++}. These data indicate a water hardness dependency of Se and Hg accumulation under acidic conditions.

Key Words: Calcium, crayfish, *Orconectes virilis*, acidification, mercury, selenium.

CHANG, P.S.S., D.F. MALLEY and J.F. KLAVERKAMP. 1982. Effets d'un pH faible et de la presence de calcium sur la bioaccumulation relative du 75_{Se} et du 203_{Hg} par sept tissus d'ecrevisses *Orconectes virilis*. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les précipitations acides augmentent de plus en plus la teneur en Se et en Hg des systèmes aquatiques. On a mesuré l'accumulation de 75_{Se} et de 203_{Hg} dans sept tissus d'écrevisses maintenus en cages placées dans des enceintes de 1m de diamètre: l'étude s'effectua au Lac 302 de la Region des lacs expérimentaux située au nord-ouest de l'Ontario. Les données pour le 203_{Hg} ont été publiées ailleurs. La demi-vie pour l'élimination du 75_{Se} dans l'eau variait de 11 à 31 jours. Les quantités de 75_{Se} dans les tissus (comptes.min.$^{-1}$.g^{-1}) augmentaient suivant l'ordre: hépatopancréas>glande du vert>intestin>ovaire>branchies>carapace>muscle abdominal. Dans la plupart des cas, l'accumulation de 75_{Se} etait plus grand à pH 5,4 qu'à pH 6,7, et elle augmentait dans un milieu acide additionné de Ca^{++}. L'acidification n'influait nullement sur le rapport 75_{Se}:203_{Hg} dans les tissues, mais ce rapport était considérablement plus élevé chez les écrevisses exposés à un milieu dont le pH était de 5,4 et de 5,0 avec addition de Ca^{++} (5 et 15mg.L^{-1}) que chez ceux en milieu neutre additionné ou non de Ca^{++}. Ces données indiquent que l'accumulation de Se et de Hg en milieu acide dépend de la dureté de l'eau.

ALKYLTIN COMPOUNDS IN THE AQUATIC ENVIRONMENT

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CHAU, Y.K., R.J. MAGUIRE and P.T.S. WONG. 1982. Alkyltin compounds in the aquatic environment. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Alkyltin compounds are extensively used in industrial and agricultural applications. The results of our studies indicating (1) methylation of tin compounds,(2) occurrence of methyltin and butyltin species in environmental samples, and (3) organotin compounds being more toxic than inorganic tins would have significant environmental consequences.

Key Words: Alkyltin compounds, methyltin, butyltin, organotin.

CHAU, Y.K., R.J. MAGUIRE and P.T.S. WONG. 1982. Les composés d'étain-alkyle dans le milieu aquatique. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les composés d'étain-alkyle sont très largement employés à des fins industrielles et agricoles. Les résultats de notre étude indiquent: (1) qu'il y a méthylation des composes de l'étain, (2) qu'on trouve des espèces méthylées et butylées dans des échantillons environnementaux et (3) que les composés organiques de l'étain, étant plus toxiques que les composés inorganiques, auraient des conséquences importantes dans le milieu.

COMPARATIVE TOXICITY OF WASTE DRILLING FLUIDS TO A CRUSTACEAN (PALEMONETES PUGIO) AND A FISH (CYPRINODON VARIEGATUS)

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CONKLIN, Philip J. and K. Ranga RAO. 1982. Comparative toxicity of waste drilling fluids to a crustacean (Palemonetes pugio) and a fish (Cyprinodon variegatus). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The acute toxicity of a series of 18 drilling fluids (muds) from an exploratory drilling operation was evaluated in tests using 28-day old juvenile sheephead minnows (Cyprinodon variegatus) and grass shrimp (Palaemontes pugio). Grass shrimp that molted during the tests were especially sensitive to the drilling muds; the 96-hr LC_{50}s (363 to 14,565 ppm mud by volume) are considerably lower than the previously reported toxicity values for drilling muds. Sheephead minnows were considerably less sensitive to the muds than were grass shrimp. Although a number of the drilling muds contained relatively high amounts of chromium, in most instances the observed toxicities did not appear to be attributable to chromium alone. However, there was a significant correlation between the amount of oil present in the muds and their toxicity to grass shrimp. (Supported by U.S. EPA Grant CR-807417)

Key Words: Drilling fluids, sheephead minnows, Palaemontes pugio, Cyprinodon variegatus, grass shrimp, chromium, oil, LC_{50} values.

CONKLIN, Philip J. and K. Ranga RAO. 1982. Toxicité relative des fluides de forage résiduaires pour un crustacé (Palaemonetes pugio) et un poisson (Cyprinodon variegatus). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On vérifie l'extrême toxicité de 18 boues de forage provenant d'un site de forage de recherche au cours d'expériences menées sur un fondule, le Cyprinodon variegatus de 28 jours, et sur la crevette Palaemonetes pugio. Les crevettes qui ont mué au cours des tests se révèlent particulièrement sensibles aux boues de forage. Les valeurs CL_{50} après 96 heures, soit entre 363 et 14 565 ppm de boue par volume, sont beaucoup moindres que valeurs de toxicité indiquées jusqu'ici pour les boues de forage. Les fondules sont beaucoup moins sensibles aux boues de forage que les crevettes. Bien que certaines des boues analysées contiennent du chrome dans une proportion assez élevée, dans la plupart des cas, ce métal ne semble pas être la seule cause de toxicité. Cependant, on note une corrélation marquée entre la quantité de pétrole contenue dans les boues et leur toxicité pour les crevettes. (Bourse de l'Agence de Protection de l' environnement des Etats-Unis CR-807417)

THE FATE OF NATURAL AND INDUSTRIAL ORGANIC COMPOUNDS ENTERING THE MICROBIAL ECOSYSTEM OF THE ATHABASCA RIVER

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COSTERTON, J.W. 1982. The fate of natural and industrial organic compounds entering the microbial ecosystem of the Athabasca River. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The Athabasca River receives hydrocarbons and organic acids from both natural and industrial sources. The former constitute a natural "cold water" extract of tar sands while the latter constitute the products of an industrial "hot water" extract of these same tar sands. We have used radioactive tracer techniques to determine the fate of hydrocarbons and of organic acids when these compounds are introduced into the Athabasca River ecosystem. Our data show, unequivocally, that the microbial degradation of these compounds within the flowing water phase of the river is very slow. However, the huge bacterial populations of the river sediments degrade both hydrocarbons and toxic organic acids at a very rapid rate. This vigorous degradation of toxic organic molecules can be used to detoxify dike drainage (patent filed) and it accounts for the phenomenal capacity of the oil-adapted bacterial population of the sediments of the Athabasca River to degrade toxic organic molecules of both natural and industrial origin.

Key Words: Tar sands, microbial degradation, oil-adapted bacteria.

COSTERTON, J.W. 1982. Cheminement des composés organiques naturels et industriels entrant dans l'écosystème microbien de la rivière Athabasca. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

La rivière Athabasca recoit des hydrocarbures et des acides organiques de diverses sources naturelles et industrielles. Les premiers sont un extrait naturel des sables bitumineux, véhiculé en «eau froide», et les seconds sont les dérivés industriels d'un extrait des mêmes sables bitumineux, obtenus par injection d'«eau chaude». À l'aide de techniques de dépistage radioactif, nous suivons le cheminement des hydrocarbures et des acides organiques après leur entrée dans l'écosystème de l'Athabasca. D'après nos observations, il est clair que la dégradation microbienne de ces composés dans l'eau courante de la rivière est très lente. Toutefois, l'énorme population bactérienne des sédiments fluviaux dégrade très rapidement les hydrocarbures et les acides organiques toxiques. Or, cette decomposition rapide des molécules organiques toxiques peut être utilisee afin de purifier les écoulements de filons d'injection (demande de brevet déposée), et cela explique aussi le fait que les bactéries des sédiments de l'Athabasca, étant adaptées à la présence de pétrole, ont une capacité extraordinaire de dégradation des molécules organiques toxiques, autant d'origine industrielle que naturelle.

SERUM SORBITOL DEHYDROGENASE (SSDH) AS AN INDICATOR OF SUBLETHAL TOXICITY

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DIXON, D.G., P.V. HODSON and K.L.E. KAISER. 1982. Serum sorbitol dehydrogenase (SSDH) as an indicator of sublethal toxicity. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Monitoring SSDH activity is a rapid method for determining short-term sublethal toxicity in rainbow trout. Levels of SSDH activity in fish were measured subsequent to intraperitoneal injection with either phenol, p-nitrophenol, p-chlorophenol or p-methylphenol at levels ranging from 0.075 to 0.75 of the 96h LD 50. In all cases a statistically significant dose-dependent increase in SSDH activity was evident 48 and 96h postinjection. Experiments to optimize the diagnostic value of SSDH showed that maximum activity occurred 48h postinjection, that freezing samples in liquid nitrogen is the only suitable method for storing serum, and that activity is affected by neither sex nor fish weight between 50 and 200 g. Serum protein levels decreased with dose in all experiments, although both the consistency and magnitude of the responses where lower than those of SSDH.

Key Words: Sorbitol dehydrogenase, fish, phenol, p-nitrophenol, p-chlorophenol, p-methylphenol.

DIXON, D.G., P.V. HODSON and K.L.E. KAISER. 1982. Sorbitol-déshydrogénase sérique: indicateur de toxicité sublétale. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

L'évaluation de l'activité du sorbitol-déshydrogenase sérique est un moyen rapide de déterminer une toxicité sublétale à court terme chez la truite arc-en-ciel. On mesure cette activité chez les poissons, après injection intrapéritonéale de phénol, de p-nitrophénol, de p-chlorophénol ou de p-methylphénol dans une proportion représentant de 0,075 et 0.75 de la valeur LD$_{50}$ après 96 heures. Dans tous les cas, on relève une augmentation statiquement appréciable de l'activité du sorbitol-déshydrogénase sérique, en fonction de la dose administrée, 48 heures et 96 heures apres l'injection. Les expériences menées en vue d'optimiser l'utilité diagnostique du sorbitol-déshydrogénase sérique ont démontré que cet enzyme atteint son point le plus actif 48 heures après son injection. Il ressort également que la congélation des échantillons au moyen d'azote liquide est le seul moyen pratique d'entreposer le sérum, dont l'activité n'est influencée ni par le sexe ni par le poids du poisson, lorsque celui-ci se situe entre 50 et 200 g. Le niveau de protéines sériques diminue avec la dose dans toutes les expériences, bien que la constance et l'amplitude de la réaction soient plus faibles que celles du sorbitol-déshydrogénase sérique.

COMPARISON OF TRACE METALS METABOLISM OF TWO MARINE CRUSTACEANS, BLUE CRAB (CALLINECTES SAPIDUS) AND AMERICAN LOBSTER (HOMARUS AMERICANUS)

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Beaufort, North Carolina

ENGEL, David W. and Marius BROUWER. 1982. Comparison of trace metals metabolism of two marine crustaceans, blue crab, Callinectes sapidus and American Lobster, Homarus americanus. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Marine invertebrates have the ability to accumulate trace metals from the environment and to sequester certain of these metals (Cd, Zn, Cu) in protein bound complexes. Two crustaceans which inhabit the coastal waters of the eastern U.S. are the blue crab and the American lobster. Through the use of animals exposed to Cd in the laboratory and in the field, we have been able to demonstrate by gel filtration chromatography that both species have specific proteins which complex not only Cd but also Zn and Cu. Initial measurements of molecular weight have been made as well as the tissue distributions of the metals. While both species possess metal-binding proteins in their tissues, there appears to be significant differences between the species with regard to molecular weight and numbers of metal-binding proteins.

Key Words: Callinectes sapidus, American lobster, Homarus americanus, Marine invertebrates, blue crab, metal-binding proteins, cadmium, zinc, copper, crustaceans.

ENGEL, David W. and Marius BROUWER. 1982. Comparaison de la métabolisation de métaux à l'état de trace chez deux types de crustacés marins, le crabe bleu, Callinectes sapidus, et le homard américain, Homarus americanus.

Les invertébrés marins sont capables d'absorber des métaux qui existent à l'état de trace dans leur environnement et d'en métaboliser certains (Cd,Zn et Cu) sous forme de complexes protéiques. Le crabe bleu et le homard américain sont deus types de crustacés des eaux littorales de l'est des États-Unis. On en expose certains spécimens à des traces de cadmium (Cd), en laboratoire et en milieu naturel, et on demontre, grâce à la chromatographie sur gel, que ces deux espèces ont des protéines spécifiques qui s'associent non seulement au cadmium (Cd) mais aussi au zinc (Zn) et au cuivre (Cu). On fait des mesures préliminaires du poids moléculaire et vérifie la distribution des métaux dans les tissus. Bien que les deux espèces soient dotées de protéines capables de s'associer aux métaux, elles semblent présenter des différences considérables quant au poids moléculaire et au nombre de ces protéines.

THE DEVELOPMENT OF AN EARLY WARNING BIOLOGICAL MONITORING PROGRAM IN NEW JERSEY

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GARIE, Henry L., Angela CANTELMO and Richard LUTZ. 1982. The development of an early warning biological monitoring program in New Jersey. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The development of an early warning biological monitoring system is described for the in situ detection of toxic pollutants. The program utilizes changes in the adenylate energy charge (AEC) system and microstructural shell growth patterns of the freshwater mollusc, Corbicula manilensis, in an attempt to detect sublethal response to stress induced by a combined industrial/domestic sewage point source discharge into the Raritan River in New Jersey. Preliminary results of AEC measured in molluscs collected from upstream sites reflect healthy populations. Molluscs samples below the point source discharge exhibit consistently lower AEC values indicative of a stressed population. Techniques used to transplant, sample and analyze the molluscs are described, and the potential for using sublethal responses of aquatic organisms as a tool in environmental risk assessment is discussed.

Key Words: Early warning biological monitoring, adenylate energy charge, mollusc, Corbicula manilensis, sublethal response, environmental risk assessment, waste water.

GARIE, Henry L., Angela CANTELMO and Richard LUTZ. 1982. Mise au point d'un programme de contrôle biologique aux fins d'alerte immédiate, au New Jersey. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On décrit dans le présent rapport la mise au point d'un système de contrôle biologique servant à la détection rapide, sur place, de polluants toxiques. Ce système doit permettre de déceler les réactions sublétales au stress causé par le déversement de rebuts domestiques et industriels en un point de la Raritan, au New Jersey, à partir des modifications observées de la charge énergétique d'adénylate et de la croissance microstructurale de la coquille d'un mollusque d'eau douce, le Corbicula manilensis. Il ressort de la vérification de la charge énergétique d'adénylate dans les mollusques pêches en amont du point de déversement que les bancs dont ils provenaient sont en bonne santé. En revanche, le taux constamment inférieur de cette charge dans les échantillons prélevés en aval du point de déversement porte à croire que les populations dont ils proviennent sont stressées. Le rapport comporte également une description des techniques employées pour la transplantation, l'échantillonnage et l'analyse, et l'on y traite de la possibilité d'utiliser les réactions sublétales des organismes aquatiques afin de déterminer le danger auquel est exposé l'environnement.

FISH AVOIDANCE OF TOXIC EFFLUENTS

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HADJINICOLAOU, J. and L.D. SPRAGGS. 1982. Fish avoidance of toxic effluents. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The behavioral change in fish exposed to an industrial effluent was assessed using a new design for a toxicity avoidance apparatus. Rainbow trout (Salmo gairdneri) were exposed to an effluent containing significant concentrations of nickel and copper. Continuous video recordings were made and the movement of fish statistically analysed to produce an avoidance curve as a function of concentration. The curve confirms that the fish avoid the effluent at a concentration considerably less than the lethal limit. The lethal limit was defined by the LC50 test in these experiments. Comparison has been made between the avoidance effect of the actual effluent and its components (copper, nickel, iron) using two different injection systems. A cluster analysis using the results of the experiments and literature information defines the role of the particular effluent to the aquatic environment.

Key Words: Fish avoidance, Rainbow trout, Salmo gairdneri, nickel, copper, iron.

HADJINICOLAOU, J. and L.D. SPRAGGS. 1982. Evitement des poissons aux effluents toxiques. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les variations du comportement de poissons, lorsque soumis à des effluents industriels, furent évaluées au moyen d'un appareil d'évitement toxique de conception nouvelle. Des truites arc-en-ciel (Salmo gairdneri) furent exposées à un effluent contenant du nickel et du chrome en concentration importante. Des enregistrements video (magnétoscopiques) ainsi qu'une étude statistique du mouvement des truites furent réalisés afin d' établir une courbe d'évitement en fonction de la concentration. L'analyse de cette dernière confirma l'hypothèse que les spécimens évitent l'effluent à des concentrations de beaucoup inférieures à la dose létale définie, pour ces expériences, par la norme CL50. Des comparaisons entre l'effet d'évitement de l'effluent sous analyse et ses composantes (nickel, fer) furent etablies pour deux différents systèmes d'injection. Une étude, basée sur les résultats obtenus et les publications antérieures, circonscrit le rôle de l'effluent à l'intérieur de son milieu aquatique.

EFFECTS OF STRESS ON THE MECHANISM OF THIOCYANATE TOXICITY IN TROUT

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HEMING, Thomas A., Elizabeth L. MEYN and Robert V. THURSTON. 1982. Effects of stress on the mechanism of thiocyanate toxicity in trout. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Effects of stress on the toxicity and metabolism of thiocyanate (SCN$^-$) were examined in rainbow trout (*Salmo gairdneri*). In non-stressed trout, SCN$^-$ had no acutely (96-h) toxic effe at water concentrations less than 10 mg.L^{-1}; concentrations greater than 100 mg.L^{-1} were required to produce 100% mortality. When trout were stressed by 30 sec of forced swimming a 96-h SCN$^-$ exposure, however, all fish died within 45 min at concentrations as low as 8 mg.L^{-1} Death of non-stressed fish is associated with an imbalance of blood ions. Thiocyanate is actively transported across the gills in direct competition with chloride ions, resulting in depletion of blood Cl$^-$ and accumulation of SCN$^-$. Death of stressed fish is characterized by an immediate loss of equilibrium, gasping and convulsions. Death of these fish may be attributed to respiratory collapse during convulsions and a stress-induced shift in the thiocyanate-cyanide chemical equilibrium, resulting in production of free cyanide *in vivo*.

Key Words: Thiocyanate toxicity, rainbow trout, *Salmo gairdneri*, cyanide, chloride.

HEMING, Thomas A., Elizabeth L. MEYN and Robert V. THURSTON. 1982. Effets du stress sur le mécanisme de toxicité du sulfocyanate chez la truite. Can. Tech. Rep. Fish. Aquat. Sci

On examine les effets du stress sur la toxicité et la métabolisation du sulfocyanate (SC chez la truite arc-en-ciel (*Salmo gairdneri*). Chez la truite qui n'est pas soumise à un stre le sulfocyanate n'a pas d'effet toxique marqué (après 96 heures) à des concentrations inférie à 10 mg.L^{-1}. Il faut une concentration supérieure à 100 mg.L^{-1} pour causer un taux de mortal de 100 p. 100. Par ailleurs, lorsque les truites sont soumises à un stress en étant forcées nager pendant 30 s après avoir été exposées au SCN$^-$ pendant 96 heures, toutes meurent après 45 mn, même à des concentrations aussi faibles que 8 mg.L^{-1}. La mort des poissons qui ne son pas soumis à un stress est liée à un déséquilibre des ions sanguins. Le sulfocyanate est ach rapidement à travers les branchies au détriment direct des ions de chlorure, ce qui entraîne baisse du Cl$^-$ sanguin et une accumulation du SCN$^-$. La mort des poissons soumis à un stress e caractérisée par la perte instantanée d'équilibre, le halètement et les convulsions. On peut l'attribuer à un affaissement du système respiratoire durant les convulsions et à un déséquil sulfocyanate-cyanure causé par le stress et entraînant la production de cyanure à l'etat libr *in vivo*.

LEAD CONTAMINATION OF FISH FROM THE SALMON RIVER, CAPE BRETON, NOVA SCOTIA

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HODSON, Peter V., Gary F. WESTLAKE and Beverly R. BLUNT. 1982. Lead contamination of fish from the Salmon River, Cape Breton, Nova Scotia. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The sediments of the Salmon River are heavily contaminated with lead as a result of lead mining and surface erosion of the ore body. The lead content of resident fish was surveyed in 1982 to determine whether this lead was biologically available and whether lead toxicity could be expected to adversely affect fish production. Lead contamination was assessed by measurement of the inhibition of erytheocyte δ-aminolevulinic acid dehydratase activity, and blood, opercular bone and whole-body lead concentrations. Preliminary results indicate that the fish are indeed contaminated.

Key Words: Lead, sediments, fish, erytheocyte, δ-aminolevulinic acid dehydratase, lead accumulation, enzyme inhibition.

HODSON, Peter V., Gary F. WESTLAKE and Beverly R. BLUNT. 1982. Contamination par le plomb des poissons de la Rivière Salmon au Cap-Breton (Nouvelle-Écosse). Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les sédiments de la rivière Salmon sont fortement contaminés par le plomb en raison de l'exploitation d'un gisement plombifère et de l'érosion en surface du corps minéralisé. La teneur en plomb de ses poissons a été mesurée en 1982 pour determiner si ce plomb était biologiquement disponible et pour savoir si sa toxicité pouvait nuire aux pêches. On a évalué l'importance de la contamination en mesurant l'inhibition de l'acide δ-aminolévulinique déshydratase dans les érythrocytes, et en déterminant la concentration de plomb dans le sang, l'opercule et tout l'organisme. Les résultats préliminaires indiquent qu'il y a reellement contamination.

A PICTURE IS WORTH 100 WORDS: IMPLEMENTING TELIDON IN AQUATIC TOXICOLOGY

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LEDUC, Gerard. 1982. A picture is worth 100 words: Implementing Telidon in aquatic toxicology. Can. Tech. Rep. Fish Aquat. Sci. 1163.

The new videotex technology now offers new means of scientific information storage and retrieval. Contrary to other conventional means of communication, Telidon offers a high speed, easy to use telecommunication service giving the user exact up-to-date information at most distant points across and between continents. In aquatic toxicology, Telidon could prove to be a most efficient communication tool for researchers, operational managers, legislators and educators providing, up-to-date information on chemical survey and bioassay data, new standards, who's doing what, and protocols for bioassays or chemical tests. Other possible applications of Telidon include: basic files and geographic distribution of toxicants, research design, emergency measures and environmental impact. A number of Telidon pages will illustrate this presentation.

Key Words: Telidon, aquatic toxicology, communication.

LEDUC, Gerard. 1982. Une image vaut 1000 mots: l'application de Telidon en toxicologie aquatique. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

La nouvelle technologie videotex offre présentement de nouvelle possibilités de stockage et de rappel d'information scientifique. Comparé aux autres moyens de communications, Telidon offre un service de télécommunication rapide, facile d'accès permettant à l'usager d'obtenir une information à jour venant de l'intérieur ou entre les continents. En toxicologie aquatique, Telidon pourrait s'avérer un des plus efficaces outils de communications pour les chercheurs, les gestionaires, les législateurs et les éducateurs, offrant une information la plus récente sur les relevés chimiques, les bioessais, les standards de référence, qui fait quoi?, les protocole de bioessais ou tests chimiques. D'autres applications de Telidon incluent: dossiers et distribution géographique de toxiques, protocoles de recherche, mesures d'urgence et impacts environnementaux. Un certain nombre de page Telidon vont illustrer cette présentation.

EFFECT OF LOW PH ON GONADAL DEVELOPMENT OF BROOK TROUT (*SALVELINUS FONTINALIS*): RESULTS FROM A STUDY ON LAKES IN THE SAULT STE MARIE AREA

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Dept. of Zoology, University of Western Ontario

ROY, Robert J.J. and W.H. TAM. 1982. Effect of low pH on gonadal development of brook trout (*Salvelinus fontinalis*) : Results from a study on lakes in the Sault Ste Marie area. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

The effects of lake pH, alkalinity and conductivity on gonadal development were evaluated on male and female brook trout taken from Sault Ste Marie area lakes in the summers of 1981 and 1982. The progress in gonadal development in each fish was determined histologically. Decreased gamete production occurred in both sexes in lakes with lower pH. It was also shown that low pH is highly correlated with a decrease in proportion of yolky eggs and an increase in the occurrence of undifferentiated eggs. The results indicate that a moderate degree of acidifidation (1 pH unit) may affect gonadal maturation in brook trout.

Key Words: Low pH, brook trout, alkalinity, conductivity, gonadal development, *Salvelinus fontinalis*.

ROY, Robert J.J. and W.H. TAM. 1982. Effets d'un pH faible sur le développement gonadique de l'omble de fontaine (*Salvelinus fontinalis*) : Résultats d'une étude des lacs de la région de Sault-Sainte-Marie. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Au cours des étés 1981 et 1982, on étudie les effets du pH, de l'alcalinité et de la conductivité des lacs de la région de Sault-Sainte-Marie sur le développement gonadique de l'omble de fontaine. Un examen histologique montre les diverses étapes du développement gonadique de chaque poisson. On constate une diminution de la production de gamètes chez les poissons mâles et femelles dans les lacs de pH faible. On constate aussi une étroite corrélation entre l'acidité des lacs et la diminution du nombre d'oeufs à embryon doublée d'une augmentation d'oeufs opaques. Les résultats obtenus au cours de l'étude indiquent que l'acidification modérée de l'eau (d'une unité pH) peut altérer la maturation gonadique de l'omble de fontaine.

PERSISTENCE AND DISTRIBUTION OF METHOXYCHLOR IN AN AQUATIC ENCLOSURE

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Guelph, Ontario

SOLOMON, Keith R., Jai Y. YOO and Narinder K. KAUSHIK. 1982. Persistence and distribution of methoxychlor in an aquatic enclosure. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Methoxychlor was applied to 125m^3 enclosures in an Ontario lake using two methods of application: complete mixing and surface treatment. Residues of methoxychlor in water, sediment and the enclosure walls were determined by gas chromatography. The difference in the distribution of methoxychlor in these systems and their possible biological implications will be discussed.

Key Words: Aquatic enclosure, methoxychlor, distribution.

SOLOMON, Keith R., Jai Y. YOO and Narinder K. KAUSHIK. 1982. Persistance et distribution du méthoxyclhore dans une enceinte aquatique. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

On répand du méthoxychlore dans des enceintes de 125 m^3, aménagées dans un lac de l'Ontario. L'application se fait de deux facons: par mélange complet et par application en surface. On analyse ensuite par chromatographie en phase gazeuse les résidus de méthoxychlore se trouvant dans l'eau, dans les sédiments et sur les parois de l'enceinte. Le rapport discute les variations observées quant à la distribution du méthoxychlore dans ces systèmes, ainsi que les répercussions biologiques qui pourraient résulter de telles distributions.

RELATIONSHIP BETWEEN STRUCTURE AND TOXICITY OF TRIARYL PHOSPHATE COMPOUNDS ON FRESHWATER ALGAE

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WONG, P.T.S. and Y.K. CHAU. 1982. Relationship between structure and toxicity of triaryl phosphate compounds on freshwater algae. Can. Tech. Pep. Fish. Aquat. Sci. 1163.

Triaryl phosphate compounds are widely used in many commerical and industrial products, notably as plasticizers, gasoline additives and hydraulic fluids. The structure and toxicity relationship of a number of these compounds on the primary productivity and reproduction of natural phytoplankton from lake water and pure algal culture was examined. In general, the triaryl phosphates were very toxic to the algae with triphenyl phosphate being the most toxic, followed by tritolyl, trixylyl and diphenyl phosphates. With tritolyl phosphate, the position of the methyl group substitution was also important in the toxicity. The "ortho" form was more toxic than the "meta," while the "para" form was relatively non-toxic to the algae.

Key Words: Freshwater algae, triaryl phosphate, phytoplankton.

WONG, P.T.S. and Y.K. CHAU 1982. Relation entre la structure des phosphates de triaryle et leur toxicité sur les algues d'eau douce. Can. Tech. Rep. Fish. Aquat. Sci. 1163.

Les phosphates de triaryle sont des composés dont l'usage est très répandu dans beacoup de produits commerciaux et industriels, notamment comme plastifiants, additifs pour l'essence et liquides hydrauliques. On a étudié la relation entre la structure et la toxicité d'un certain nombre de ces composés sur la productivité primaire et la reproduction du phytoplancton naturel des eaux de lacs et de cultures d'algues pures. En général, les phosphates de triaryle étaient très toxiques pour les algues, le· phosphate de triphényle l'étant davantage que les autres, puis, dans l'ordre, les phosphates de tritolyle, de trixylyle et de biphényle. Dans le cas des phosphates de tritolyle, la position du groupement méthyle influait aussi beacoup sur la toxicité: Le composé "ortho" était plus toxique que le "méta", alors que le "para" était relativement non toxique pour les algues.

LEGAL ASPECTS OF AQUATIC TOXICITY
Rv. Great Canadian Oil Sands: A Case Study

by

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In the enforcement of environmental quality control standards, it is imperative scientists and lawyers develop a close liaison so that the former knows what is needed to prove a case and the latter, what evidence can be obtained by the scientists. The Great Canadian Oil Sands (GCOS) (Rv. Great Canadian Oil Sands), District Court of Alberta, January 10, 1978 case has proven to be a case in point. It left Alberta lawyers and scientists confused as to the required evidence for proof of charges in the province under the Fisheries Act.

In 1976 Great Canadian Oil Sands was prosecuted for violations under the Fisheries Act. It was alleged that GCOS had discharged 400,000 gallons per day of a substance containing cadmium and vanadium from pipes in the dykes of the GCOS tailings pond. Section 33(2) of the Fisheries Act makes it an offence to DEPOSIT A DELETERIOUS SUBSTANCE into waters FREQUENTED by fish.

In order to prove the charge, water samples were taken and bioassay tests performed with brook stickleback and rainbow trout. The Crown also introduced expert testimony of a fish toxicologist and fish biologist to prove the deleterious effect of the substance on fish.

The trial judge held that there was no evidence of dead fish along the river and that actual damage to fish was necessary before there was an offence under the Act. Therefore, GCOS was acquitted.

This ruling was appealed. The appellate judge found the company not guilty for a number of reasons including:

1. The Crown failed to produce evidence of chemical composition of the effluent. Therefore, the effluent itself could not be found to be toxic to the fish.

2. There was no pathological evidence showing how the fish used in the bioassay died.

3. There was no evidence of adverse effects of fish found in the waters.

4. Toxic substances may already have been in the water naturally because the river flows through outcropping of tar sands which release natural oil slicks. Even if the GCOS discharge reached the river, it may not necessarily have impaired the water quality.

5. Brook stickleback and rainbow trout are rare in the river; therefore, they could not be said to "frequent" the river as was required by the Act.

6. The dykes discharged the effluent onto land which meandered towards the river and there was no evidence brought forth to prove that the discharge actually reached the river itself.

The general conclusions which appear to be supported by the GCOS decision with regards to evidence for proof of an offence under S 33(2) include:

1. It may be necessary to have proof of a deleterious effect on fish which are common in the water at the time of the deposit;

2. It may not be essential to show that dead fish were found along the river but such evidence would be useful if available. The presence of dead fish alone, is not sufficient to prove a substance is deleterious;

3. Bioassay tests are not essential for proof of the deleterious effect of a substance on fish; the appellate judge considered them circumstantial evidence only. If bioassay tests are to be performed it is likely essential that the fish used in the test be ones commonly found in the waters in question.

Analysis

The GCOS decision is inconsistent with decisions from similar prosecutions in British Columbia. In Rv. MacMillan Blodel (Alberni) Ltd. the judge held it was sufficient for the Crown to show the substance was deleterious to any species of fish regardless of whether it was a species found in large numbers in that river. This case also held that the Crown does not need to show that the water has been rendered deleterious by the deposit of the substance but rather that the substance itself is deleterious. This means that the deposit of one drop of a substance that is deleterious to fish in the river is an offence even if the actual water is not rendered deleterious.

In another case heard in British Columbia, the judge ruled that it was only necessary for the Crown to prove that some fish were in the river at some time of the year. There need not be fish present in large numbers or at the time of the deposition. This is inconsistent with the GCOS decision wherein it was held to be necessary to show deleterious effect on fish common in the water.

The GCOS decision is an important precedent for the interpretation of S.33(2) of the Fisheries Act in Alberta. It is also a very useful lesson for lawyers and scientists as to the type of evidence required by Alberta Courts.

However, there are many unanswered questions raised as a result of the interpretation given to this section by Alberta Courts and B.C. Courts. These include:

1. Is it necessary to take fish from the waters in question to perform bioassays? May the biologist use species found in the river? May the biologist use standard laboratory experimental fish?

2. Must the fish harmed be found in large numbers or is it sufficient that they are found in the water?

3. Do the fish harmed have to be found at the location of the spill at the time of spill?

4. Is it necessary that the water be made deleterious to fish or is it sufficient to show the substance itself is deleterious?

Only further prosecutions will result in clarification of these issues so that government and industry will know how the law is to be interpreted in Alberta.

La Reine contre Great Canadian Oil Sands: Étude de cas

Dans l'application des normes de contrôle de qualité en matière d'environnement, il est essentiel que les scientifiques et les avocats entretiennent des liens étroits, afin que les premiers connaissent les preuves requises pour soutenir une cause et les seconds aient une idée des preuves que peuvent donner les scientifiques. La cause entendue par la cour de district d'Alberta, le 10 janvier 1978 (la Reine contre Great Canadian Oil Sands) reflète justement ce besoin. Les avocats et les scientifiques se sont trouvés désémpares quant aux preuves nécessaires pour soutenir le motif d'accusation porté en vertu des dispositions de la Loi sur les pêcheries, en Alberta.

En 1976, on a intenté une poursuite contre la GCOS pour motif d'infractions à la Loi sur les pêcheries. En effet, la GCOS aurait devérse chaque jour 400 000 gallons d'une substance contenant du cadmium et du vanadium dans les tuyaux menant aux digues de son étang de résidus. L'article 33.(2) de la Loi sur les pêcheries établit qu'il est interdit de<<déposer dans les eaux fréquentées par le poisson... toute ...substance ou chose délétère>>.

Dans le but de faire la preuve du chef d'accusation, on a pris des échantillons d'eau et on a effectué des analyses sur des épinoches à cinq épines et des truites arc-en-ciel. La Couronne a également présenté les témoignages d'un toxicologue et d'un biologiste, spécialistes en ichtyologie, afin de prouver qu'il y avait eu déversement de substance délétère.

Le juge d'instances décida qu'on n'avait pas de preuve qu'il y ait en des poissons morts le long de la rivière et qu'il fallait prouver que les poissons avaient été atteints pour qu'il y ait infraction à la loi. Par conséquent, la GCOS a été acquittée.

On en a appelé de cette décision. Le juge du tribunal d'appel a reconnu la société non coupable pour diverses raisons, dont les suivantes:

1. La Couronne n'a pas réussi à présenter des preuves de la compostion chimique des effluents. Par conséquent, il n'était pas possible d'affirmer que les effluents avaient causé la mort des poissons.

2. Il n'y avait pas de preuve fondée sur les tests pathologiques pour démontrer comment les poissons étaient morts lors de l'essai biologique.

3. On n'avait pas prouvé les effets néfastes des effluents sur les poissons pris dans ce cours d'eau.

4. Il se peut que des substances toxiques se soient déjà trouvées dans l'eau, et ce naturellement, étant donné que la rivière traverse des gisements de sables bitumineux d'où s'échappent des coulées d'huile. Même si les effluents de la GCOS avaient atteint la rivière, cela ne signifie pas pour autant que la qualité de l'eau en ait souffert.

5. Les épinoches à cinq et les truites arc-en-ciel sont rares dans la rivière. Par conséquent, on ne peut pas dire que ces poissons fréquentent la rivière, tel que le précise la loi.

6. Les digues laissent s'écouler les effluents sur le sol, et l'on n'a pas prouvé que les effluents eaux-mêmes ont atteint la rivière.

Voici certaines conclusions générales qui se détachent de la décision rendue dans le cas de la GCOS en ce qui concerne la présentation de la preuve d'infraction en vertu de l'article 33.(2):

1. Il peut être nécessaire d'avoir des preuves de l'effet délétère des effluents sur les poissons qui sont communs dans la rivière au moment de la décharge toxique.

2. Il n'est peut-être pas essentiel de démontrer que des poissons morts ont été trouvés le long de la rivière. Ces preuves pourraient toutefois se révéler utiles si on les aviat. La présence de poissons morts ne suffit pas, à elle seule à prouver qu'une substance est délétère.

3. Les essais biologiques ne sont pas essentiels à la preuve des effets délétères d'une substance sur les poissons. En effet, le juge d'appel a estimé qu'il ne s'agissait là que de preuves obtenues par présomption. S'il faut procéder à des essais biologiques, il est probablement essentiel que les poissons testés soient des poissons que l'on retrouve communément dans les eaux en question.

Analyse

La décision rendue dans le cas de la GCOS ne cadre pas avec celles qui ont été rendues dans des cas semblables en Colombie-Britannique. Dans le cas de la *Reine contre MacMillan Bloedel (Alberni) Ltd.*, le juge décida qu'il suffisait à la Couronne de prouver que la substance était délétère pour n'importe quelle espèce de poisson, que ce soit un poisson abondant dans ce cours d'eau ou non. Il ajouta que la Couronne n'avait pas à prouver que l'eau était devenue délétère par suite du déversement de la substance, mais seulement que la substance elle-même était délétère. Cette décision laisse supposer que le déversement dans une rivière d'une seule goutte d'une substance délétère pour les poissons constitue une infraction, même si l'eau n'en devient pas pour autant délétère.

Dans une autre cause, entendue également en Colombie-Britannique, le juge a déclaré qu'il suffisait à la Couronne de prouver qu'il y avait des poissons dans la rivière à un moment de l'année. Il n'était pas nécessaire qu'il y ait un grand nombre de poissons ou même qu'il y ait des poissons dans l'eau au moment du déversement. Cette décision ne rejoint pas la décision rendue dans le cas de la GCOS, selon laquelle il aurait fallu prouver l'effet délétère du déversement sur les poissons que l'on retrouvait communément dans le cours d'eau.

La décision rendue dans le cas de la GCOS constitue un précédent important quant à l'interprétation de l'art. 33.(2) de la *Loi sur les pecheries* en Alberta. De plus, cette décision donne aux avocats et aux scientifiques une idée très nette des preuves exigées par les tribunaux de l'Alberta.

Néanmoins, l'interprétation de cet article par les tribunaux de l'Alberta et de la Colombie-Britannique laisse certaines questions sans réponse, entre autres:

1. Est-il nécessaire de prélever des poissons des eaux en question pour faire les essais biologiques? Le biologiste peut-il employer des espèces qui se retrouvent dans la rivière? Peut-il employer des poissons qui servent généralement aux expériences en laboratoire?

2. Faut-il qu'un grand nombre de poissons soient atteints ou suffit-il qu'il y ait des poissons atteints?

3. Faut-il retrouver des poissons atteints sur le site et au moment mêmes du déversement?

4. Est-il nécessaire de prouver que l'eau est devenue délétère pour le poisson ou suffit-il de démontrer que la substance elle-même est délétère?

Ces questions ne seront tirées au clair que par suite d'autres procès; les gouvernements et les industries seront alors en mesure de savoir quelle est l'interprétation que l'on donne à la loi en Alberta.

SUMMARY OF WATER QUALITY CRITERIA WORKSHOP SESSIONS

by

M.R. Speyer

Panel Participants: M.R. Speyer, Noranda Research Centre, Pointe Claire, Quebec
M.C. Taylor, Environment Canada, Water Quality Branch, Ottawa, Ontario
G.R. Craig, Ontario Ministry of the Environment, Water Resources, Rexdale, Ontario.
R.C.H. Wilson, Environmental Protection Service, Dartmouth, Nova Scotia.

The water quality criteria (objectives) workshop discussion sessions were held on two consecutive days. To initiate the first session, panel members each briefly presented their specific views and concerns on water quality objectives. These are briefly summarized as follows:

M.C. Taylor: Margaret has been and still is responsible for the publication "Guidelines for Surface Water Quality". The purpose of the document is to provide water managers with information regarding toxic effect of chemicals found in water that could have five different designated uses. The recommended objectives should be used for developing site specific objectives taking the local type of water into consideration (e.g.,hard-soft), socio-economic factors, the uses to be made of the local water, whether high natural levels of the particular constituent exist, bioaccumulation, etc. Finally, analytical methods and objectives should be compatible as it is useless to recommend a level of chemical that cannot be detected routinely.

G.R. Craig: Regulatory agencies are increasingly faced with demands by the private sector to justify water quality objectives in light of more economically demanding times.
Development of water quality objectives previously involved locating the most sensitive biological response and lowest concentration cited in the literature as the endpoint. Now that data bases on a toxicant's activity are more extensive, this allows the development of objectives that describe different allowable concentrations of a contaminant in different receiving waters yet providing the same level of biological protection. While the development of future objectives is being impeded by budgetery restraints, a process by which industry could review prospective objectives should be initiated.

R.H. Wilson: In Atlantic Canada, where Section 33 of the Fisheries Act is administered by Environment Canada, the federal approach, too, is towards the specification of effluent standards based on site specific water quality objectives (provided that existing regulations are met). The influence of metal speciation and the more general problem of biological availability continue, however, to dictate a conservative stance.

In the coastal environment, the approach of protecting the most sensitive use and the most sensitive species frequently leads to effluent controls whose purpose is to protect shellfish and/or the associated fishery. For example, all the present fishery closure zones in Atlantic Canada are based on the contamination of molluscs or crustaceans by microorganisms or chemicals.

Inability to predict uptake rates of metals and organics in specific estuaries means that the development of water quality objectives must necessarily be somewhat arbitrary, especially in situations where bioaccumulation is important.

M.R. Speyer: Technologically derived effluent standards are an integration of available control systems, treatment costs and an accepted but limited effect; water quality objectives are derived from unadulterated science. While the latter should not be compromised by the limitations of pollution control technology, an unprejudiced review should be initiated to examine the manner by which objectives are developed. The almost exclusive use of laboratory derived toxicity results is questioned in the case of many of the heavy metals where total concentrations do not necessarily reflect toxic levels. The review of some water quality objectives, especially those developed by the U.S.-EPA (1980) for the protection of marine aquatic organisms, suggests a disregard for the bioaccumulation potential of some contaminants.

SESSION I

To set the ground-work for the group discussion period, dictionary definitions were presented for the terms criteria, objective and regulation. These definitions were presented as:

Criterion - a standard on which judgement may be based.
Objective - an aim or end of action.
Regulation - an order issued by an executive authority of a government and having the force of law.

The audience appeared less concerned with the actual definitions than their application in the overall development of water quality objectives. A discussion on the purpose of objectives and regulations was then initiated. Generally, the consensus was that a receiving water objective served as a basis for setting effluent regulations. The majority of the workshop participants felt that, for a lack of a better approach, the use of laboratory toxicity test results for setting objectives was justified. Furthermore, the use of lowest effect concentrations for a very sensitive test organism in developing receiving water objectives was also believed to be a valid approach by most participants.

SESSION II

R. Wilson started the second session of the workshop with a case study which he used as an example to demonstrate how receiving water objectives were used to set effluent regulations. Factors that were implicated in the final decision were such items as water flow, bioaccumulation potential of the contaminants and protection of a local fisheries resource, such as an oyster fishery, having regard to site specific conditions. Fluctuations in the quality and quantity of effluent river discharges complicate the determination of effluent standards which accurately reflect the need for control based on water quality objectives. The precision of such standards is, as a result, much less than that of the underlying toxicology.

The subjective approach to setting some water quality objectives was discussed raising the objectives (protection of aquatic life) for selenium as an example. The Ontario Ministry of the Environment (1979) and the International Joint Commission (1976) set Se objectives of 100 and 10 ug/L respectively even though the rationale sections for the two objectives were identical.

The restrictive U.S. EPA approach in setting ambient water quality criteria was also briefly discussed. The present approach by the U.S. EPA disregards any toxicity information that does not provide a 96-h LC50 or was not derived from a life-cycle or partial life-cycle study. The bioaccumulation of heavy metals was not considered in most cases and resulted in some criteria (especially marine) being set too high to prevent significant bioconcentration of contaminants in commerically important organisms from occurring.

That many objectives (both Canadian and American) have been set at or below detection limits of routinely available measuring equipment was recognized to be a problem. Most participants felt that objective concentrations of contaminants should be easily measured by technology that is commonly available to most enforcement agencies and other groups involved in routine monitoring.

SUMMARY

Judging by comments from the workshop participants and the panel members, it appears that the development of water quality objectives is an issue of concern. Some highlights of the discussion periods are as follows.

It was noted that different agencies will use different approaches to establish objectives and arrive at different values depending on the desired level of protection. It would therefore in the future be expected to observe more conflicting endpoints and less agreement between agencies as new objectives are developed.

The majority of participants recognized existing objectives as being "better than nothing" for the protection of a particular water use. These objectives provide a reference point for further discussions between government and industry in setting final site-specific receiving water objectives. It was also suggested by one of the panel members that industry become more involved in the review process and actually participate in the development of objectives and regulations. This would result in industry and government becoming collaborative partners in environmental protection to meet their social responsibilities as a part of the community.

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Les séances de discussion en atelier sur les critères ou objectifs de la qualité de l'eau ont duré deux jours. On a entamé la première séance en demandant aux experts de présenter brièvement leur point de vue quant aux objectifs de qualité des eaux. Voici un apercu de ces présentations:

M.C. Taylor: M$^{\text{me}}$ Taylor est responsable des Lignes directrices sur la qualité des eaux de surface, dont la 1$^{\text{ère}}$ édition a paru en 1979. Ce document donne aux gestionnaires l'information voulue sur les effets toxiques des produits chimiques que l'on retrouve dans l'eau qui pourrait avoir cinq utilisations. Il est possible, en tenant compte de la composition de l'eau dans la région, de se servir des objectifs recommandés afin d'élaborer des critères appropriés au site:

eau douce ou dure, facteurs socio-économiques de la région, usage qu'on fait de l'eau, forte concentration naturelle d'une composante donnée, accumulation biologique, etc. Par ailleurs, il faut que les objectifs soient compatibles avec les méthodes d'analyse, car il est unutile de recommander un seuil d'acceptabilité pour un produit chimique donné, s'il est impossible d'en déceler facilement la présence.

G.R. Craig: Étant donné la conjoncture économique, les agences de règlementation doivent de plus en plus justifier auprès du secteur privé les objectifs de qualité des eaux. Auparavant, l'élaboration de tels objectifs supposait qu'on accepte, comme exemple de cas limites, la réponse biologique la plus sensible et la concentration la plus faible relevées au cours des études. Grâce à des bases de données plus complètes sur les effets de divers produits toxiques, ont peut maintenant fixer des objectifs qui spécifient des concentrations différentes pour un produit toxique selon les eaux de réception, tout en accordant le même niveau de protection biologique. Les restrictions budgétaires actuelles empêchent l'élaboration de nouveaux objectifs, mais il faudrait quand même entreprendre un processus permettant à l'industrie d'examiner les objectifs proposés.

R.H. Wilson: Dans les Maritimes, où l'application de l'article 33 de la Loi sur les pêcheries relève d'Environnement Canada, le gouvernement fédéral a tendance à préciser les normes relatives aux eaux résiduaires en se fondant sur des objectifs très précis (tout en satisfaisant aux règlements en vigueur). L'influence de la combinaison des métaux à d'autres substances et le problème de nature plus générale que représente la disponibilité biologique continuent à dicter une attitude peu flexible.

Dans un environnement marin, l'approche visant à protéger les espèces les plus sensibles mène généralement à l'imposition de contrôles des eaux résiduaires qui permettent de protéger les crustacés ou l'industrie de pêche locale. Par exemple, certaines zones de la région de l'Atlantique ont dû être fermées à la pêche par suite de la contamination des mollusques ou des crustacés par des microorganismes ou des produits chimiques.

L'incapacité de prédire les taux de pénétration des composés organiques dans les estuaires signifie que l'élaboration d'objectifs de qualité des eaux sera nécessairement arbitraire, surtout dans les situations où le taux d'accumulation biologique est important.

H.R. Speyer: Les normes visant les eaux résiduaires industrielles résultent de l'intégration des meilleurs systèmes de contrôle existants, du coût du traitement et d'un effet qui est reconnu, même s'il est limité: les objectifs de qualité des eaux sont tirés d'expériences scientifiques. Ces objectifs ne devraient pas être limités par la technologie dans le domaine du contrôle de la pollution; il faudrait analyser impartialement la facon dont on élabore les objectifs. Dans le case de nombreux metaux lourds, on met en doute l'emploi quasi exclusif des résultats de tests de toxicité effectués en laboratoire, parce que, dans ce cas la concentration totale ne reflète pas nécessairement le niveau de toxicité. L" analyse de certains objectifs visant la qualité des eaux, particulièrement des objectifs établis pour l'E.P.A. américaine en 1980 en vue de protéger les organismes marins semble ne pas tenir compte du potentiel d'accumulation biologique de certains contaminants.

Séance I

Afin de jeter les bases de la discussion, on a présenté des définitions tirées de dictionnaires pour les mots suivants: Critère, objectif et règlement:

Critère - principe auquel on se réfère pour

Objectif - but précis que se propose l'action

Règlement - acte législatif qui émane d'une autorité autre que le Parlement; décision administrative qui pose une règle générale valable pour un nombre indéterminé de personnes ou de situations.

Les participants semblaient se préoccuper moins des définitions comme telles que de leur application à l'élaboration globale d'objectifs de qualité des eaux. Il s'ensuivit alors une discussion sur le rôle des objectifs et des règlements. De facon générale, on estimait que l'objectif relatif aux eaux réceptrices formait la base des règlements visant les eaux usées. La plupart des participants estimaient que, faute d'une meilleure approche, il était justifié d'employer des tests de toxicité en laboratoire pour établir des objectifs. Par ailleurs, la plupart pensaient que l'utilisation du concept de la concentration effective la plus faible pour un organisme très sensible en vue d'élaborer les objectifs relatifs aux eaux réceptrices constituait une approche valable.

Séance II

R. Wilson a marqué le pas de l'atelier en faisant un survol d'une étude de cas qui lui a servi d'exemple pour démontrer la facon dont on se sert des objectifs relatifs aux eaux réceptrices pour élaborer des règlements sur les eaux usées.

Compte tenu du fait qu'il faut également prendre en considération les conditions étroitement liées au site, voici quelques-uns des facteurs qu'on a retenus dans la décision finale: volume d'écoulement, potentiel d'accumulation biologique des contaminants et protection des industries de pêche locales, comme l'ostréiculture. Les fluctuations qualitatives et quantitatives des eaux usées dans les rivières rendent plus difficile l'élaboration de normes précises qui puissent refléter exactement le niveau de contrôle requis pour satisfaire aux objectifs de qualité des eaux. En règle générale, ces normes sont moins précises que les normes établies en laboratoire de toxicologie.

L'approche subjective qui sert à l'élaboration de certains objectifs de qualité des eaux a été abordée lors d'une discussion fondée sur les objectifs relatifs à la teneur en sélénium (établis pour la protection de la vie en milieu aquatique). Le ministère de l'Environnement de l'Ontario, en 1979, et la Commission internationale mixte, en 1976, ont respectivement fixé un objectif de 100 et de 10µ g/l, malgré un raisonnement identique.

L'approche restrictive adoptée par l'E.P.A. aux États-Unis vis-à-vis de l'élaboration de critères pour la qualité du milieu aquatique ambiant a également fait l'objet d'une brève discussion. Cet organisme néglige à l'heure actuelle tout renseignement sur la toxicité qui ne touche pas une LC 50 après 96 heures ou qui ne provient pas d'une étude d'un cycle vital ou d'une partie d'un cycle vital. Dans la plupart des cas, l'accumulation biologique des métaux lourds n'a pas été étudiée, ce qui a mené à l'élaboration de critères trop strictes pour prévenir une concentration biologique importante de contaminants dans des organismes à grande valeur commerciale, surtout en milieu marin.

Le fait que (aux É.-U. et au Canada) de nombreux objectifs aient été fixés en fonction du matériel de mesure généralement disponible ou encore en-decà du niveau mesurable à l'aide de ce matériel constitue effectivement un problème. La majorité des participants estimaient que la concentration des contaminants devrait pouvoir être mesurée facilement à l'aide des techniques dont dispose actuellement la plupart des agences et des autres groupes qui font le contrôle de facon régulière.

RÉSUMÉ

Si l'on en juge d'après les observations des experts et des participants, il semblerait que l'élaboration d'objectifs sur la qualité des eaux constitue un problème réel. Quelques-uns des faits saillants des discussions apparaissent ci-dessous.

On note que les diverses agences compétentes se servent d'approches différentes pour dresser des objectifs et fixent des valeurs différentes, selon le niveau de protection voulu. On peut donc s'attendre à ce qu'il y ait plus d'objectifs contradictoires et moins de cohérence entre ces agences au fur et à mesure qu'on élabore de nouveaux objectifs.

La plupart des participants reconnaissent que les objectifs actuels valent mieux que rien, car ils constituent un seuil qui pourrait servir de base aux discussions des gouvernements et de l'industrie en vue de l'élaboration des objectifs pertinents au site et relatifs aux eaux réceptrices. L'un des experts a proposé que l'industrie s'intéresse davantage à la révision des objectifs et des règlements et y participe de facon plus active. Il en résulterait la collaboration de l'industrie et des gouvernements, qui satisferaient ainsi à leurs responsabilités sociales vis-à-vis de la collectivité environnante.

AQUATIC TOXICOLOGY - WHERE DO WE GO FROM HERE?

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The LC_{50} should not be regarded as the epitome of aquatic toxicology, which has accumulated a considerable body of knowledge on the whole-body response to xenobiotics. The reliance on short term bioassays has emphasized the immediate effects of exposure over chronic, or delayed, effects. The lack of a large body of knowledge on anatomo-physiological responses is a sign of this skewed approach. There needs to be more work on evaluation and prediction of adverse effects, particularly, in the field of aquatic toxicology. Improved communication between aquatic and other areas of toxicology would remove mutual misconceptions. To answer the question in the title of this paper, it is necessary to ask: is aquatic toxicology a biological discipline or a multi-disciplinary field?

Key Words: Aquatic toxicology, xenobiotics, bioassays.

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On ne devrait pas considérer le CL50 comme valeur par excellence en toxicologie aquatique, malgré l'importance des connaissances acquises dans ce domaine sur la réaction d'un corps sain aux agents extérieurs. L'utilisation de ces analyses biologiques à court terme a mis l'accent sur les effets immédiats du contact avec un agent étranger au détriment des effets chroniques ou à terme. L'insuffisance des données sur les réactions anatomo-physiologiques démontre bien que l'approche est biaisée. Davantage de travaux sur l'évaluation et la prévision des effets nocifs sont nécessaires, notamment en toxicologie aquatique. Nous avons donc tout intérêt à améliorer les échanges entre la toxicologie aquatique et les autres domaines de la toxicologie afin d'éliminer les malentendus de part et d'autre. Pour répondre a la question du titre de cet article, il faut se demander: la toxicologie aquatique est-elle une discipline biologique ou un domaine multi-disciplinaire?

AQUATIC TOXICOLOGY - WHERE DO WE GO FROM HERE?

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As a veterinarian I note that we are rarely summoned to assist at normal births except in the case of expensive racehorses. Usually, we are summoned to assist at the difficult cases where the birth process has gone awry. Thus our experience tends to pertain to the abnormal birth process. Because of this, male veterinarians are frequently very uptight when their wive are giving birth. They anticipate the worst because that is what their experience would teach them. Toxicology is the study of adverse effects of chemical and other agents on living organisms. Just as our expectant veterinarian must remember that most parturitions proceed without complications, so must the toxicologist remember that most living organisms have responses that fall into a spectrum of normality. The abnormal or adverse responses are an aberration of that spectrum. The toxicologist and our expectant veterinarian must consider the adverse reaction in the context of normality.

When one considers that the same species can be found in the Barren Lands of Canada, the Sahara of Africa, the Arizona desert and the rain forest of Brazil, and is carnivore, omnivore or herbivore, we are reminded that many organisms adapt to a wide range of environments. This is important because we have to be able to distinguish between physiological responses (normality) and pathological (adverse) effects and, further, to determine whether they are significant or not, or reversible or not. The species I referred to above is a terrestrial mammal that, if you reject the Hypothesis of Goose-Berry, spent the first 36 weeks of existence as an aquatic mammal. I am, of course, referring to man (Homo sapiens). Man, for better or worse, is the prime focus for much of the current work in toxicology. And I would like to look at some of the similarities and differences between aquatic toxicology, whatever that may be, and mammalian toxicology, whatever that may be. If we exclude those members of the human race who are studying the effects of inhalation of the products of combustion of Nicotiana tabacum, most toxicology is conducted on animal models with extrapolation of these findings to man. There are some characteristics of aquatic and mammalian toxicology that it would be helpful to further explore.

Aquatic Toxicology	Mammalian Toxicology
1. Protection of populations of many and diverse species.	1. Protection of individuals of one species.
2. Margin of error not significant, little societal implication in being wrong.	2. Margin of error significant, error unacceptable to society.
3. Can test species of concern.	3. Animal models.
4. Species of concern not always known.	4. Species of concern known.
5. Extrapolation uncertain.	5. Extrapolation uncertain.
6. Regulatory requirements.	6. Regulatory requirements.
7. Test systems unstable.	7. Test systems stable.

Let us now explore how these two branches of toxicology would examine for toxicity an industria product being released into the aquatic environment. As I understand it, the aquatic toxicologist might examine this industrial product by exposing rainbow trout (Salmo gairdneri) for 96 hours to known concentrations of this product in static and flow-through toxicity tests. The product might be tested using one of the bioassays based on quenching of luminescence (Photobacterium phosphorescens), loss of mobility (Spirillum volutans), or daphnids. Surveys may be conducted of invertebrate and fish populations. An aquatic model ecosystem may be studied for determination of bioconcentration and checked by field observations. These studies would give us the following information:

a. 96 h LC50 Salmo gairdneri
b. 5 m EC50 Photobacterium phosphorescens
c. 5 m MEC50 Spirillum volutans
d. 48 h LC50 Daphnia magna
e. bioconcentration ratios
f. maximum allowable toxicant concentration (MATC)
g. changes in invertebrate and vertebrate populations

In the same example, what might the mammalian toxicologist do? Before conducting any toxicity testing, the toxicologist would like to define the industrial product. What chemical or chemicals are present in this industrial product and in what quantities? In some cases it would be helpful to know the species present. We are all aware of chromium VI, but what of arsenic, copper, mercury or selenium. Valency, inorganic or organic forms,and isomerization will affect toxicity. Will chemical reactions occur when the industrial product enters the aquatic environment? At the Alberta Environmental Centre we are conducting research on sulphur emissions which leave the stack primarily as sulphur dioxide. Subsequent reactions convert some of this gas into sulphur acids and particulates, thus changing the potential toxicity of this emission (or industrial product). If one knows the chemical compounds in the product, it is possible to predict some possible effects. Konemann (1981a,b,c) proposed the Mixture Toxicity Index for dissimilar action mixtures; Anderson and Weber (1975) the Concentration Addition, for simple like-acting mixtures. Unless we can define the product in chemical terms, whether as a pure single compound or a mixture of known aliquots of compounds, it is impossible to determine dose as opposed to exposure concentration. Which constituents of a mixture cause the adverse effects? Knowledge of the chemical constitution of this product might enable the toxicologist, whether aquatic or mammalian, to predict which body systems might be affected, or which species might be affected.

The purpose of the acute toxicity tests, in whatever species, and whatever environment, should be threefold:

1) to identify clinical manifestations of acute toxicity;
2) to give dose-ranging guidance for other tests;
3) to establish LC_{50}, LD_{50}, EC_{50} or ED_{50} values for comparison with other substances.

It should be recognized that this parameter is variable and not easily reproducible between laboratories. These values are means to an end, not the end. Although, of course, for the dead animal it is indeed the end. Remember that acute studies constitute the initial work in studying the toxicity of this product. One would hope that the route of administration is correlated with the route of exposure. Whales and sharks might differ in the route of exposure because one is air-breathing. What is the relationship between exposure concentration and response. Are there differences in response between species? Most of us take aspirin for headaches; should you give the same drug to your cat you may have the headache explaining to your spouse why your pet died. This is because the cat has difficulty in metabolizing and excreting the active ingredient contained in one tablet. Clinical signs, behavior, times of onset and recovery, gross pathology and histology will give some indication of target organs and body systems affected. In addition to observation of test animals, our mammalian toxicologist would request necropsy for all animals that died during the test and of all survivors. If the purpose is to gain some understanding of the adverse effects then use sufficient animals, for example 10 per exposure level.

If one is solely interested in determining that dose which will kill 50 percent of the exposed population, then one can get almost as good an estimate with two animals per dose. The point that I wish to make is that it is a waste of resource to refine to the nth degree a number which is inherently variable. It would be a better use of resources for the acute toxicity test to indicate the target systems or organs and the adverse reactions that might be expected. The LC_{50} value is an indication of relative toxicity and is a meaningless number, in relation to the real world, unless further information is available. Both the aquatic and mammalian toxicologist conduct their acute toxicity tests with whole, live animals and, within the context of this workshop, I include invertebrates and vertebrates as animals. For the mammalian toxicologist there is a growing and significant pressure to reduce the number of animals used for research purposes. Indeed, over the past decade the number of animals used for research purposes in North American has declined. However, we should remember that the majority of animals are used for regulatory requirements. Considerable intellectual encouragement and financial support is now available for alternative means of screening for regulatory purposes. I would ask you whether it is any less rational to conduct bioassays on *Photobacterium phosphorescens* or *Spirillum volutans* when we are concerned with the aquatic environment than it is to use *Salmo gairdneri*, *Rattus norvegicus* or *Canis familiaris*, when the focus of our concern is man and the terrestrial environment.

What we have been doing so far is to follow a tier approach. The first tier was to define the material to be examined; assessment of exposure; and examination of the acute toxicity of the industrial product. At this juncture both the aquatic and the mammalian toxicologist are faced with major decisions. Let us assume that the product did not immediately kill or cause observable adverse reactions in the several species tested in the acute toxicity test. The mammalian toxicologist would then proceed to a second tier to conduct metabolism and toxicokinetic studies. The acute studies gave an approximation of dose ranges, and the metabolism and kinetic studies would extend this information and have three purposes:

1) to ascertain qualitatively and quantitatively the action of the body on the industrial product and the degree of species variation in this regard;
2) to define the kinetic parameters for absorption, disposition, metabolism and elimination;
3) to give guidance in design of extended studies, for example; species, dose, route and frequency of administration, duration and specific tests.

One is aiming to establish a "metabolic fingerprint" and kinetic parameters. The metabolic fingerprint will facilitate a detailed analysis of metabolic pathways and metabolites; will determine if there are changes in the metabolic pathway with changes of dose; if there is repeated exposure is there enzyme induction which would not be seen following a single dose; are there species variations. The kinetic parameters will enable the appropriate mathematical model to be developed to describe the observations and will ascertain the dose and other conditions under which a steady state is attained and the various metabolic, transport, excretory and other processes become saturated. At this point I have to confess that this is the ideal approach.

It cannot be emphasized too strongly that acute toxicity tests will not detect chemicals whose effect requires passage of time before expression. Neither a 96-hour, nor a 14-day, acute toxicity test would identify asbestos, diethylstilbestrol, or benz-a-pyrene as very toxic compounds. These short tests might identify aflatoxin as an acute hepatotoxin but would miss the neoplasia which will develop later. In fact, one might hypothesize that death, although a dramatic end-point for an individual of any species, may be an insignificant end-point from the perspective of a population. World War I may be regarded as a toxic event in terms of human epidemiology since it decimated males of reproductive age in several countries of Europe. This decimation was compounded by a world-wide epidemic of a virulent influenza virus. Sixty years later we are very concerned at the growth of the human population. This is not to deny that there were local effects but on a global basis these were a blip. Death as a biological end-point may be contrasted to a mutagenic agent which produces deleterious genetic effects in subsequent generations. It is a chilling thought to remember that some 10 percent of all human disease may have a genetic component; whether chromosomal aberration (Downe's syndrome), single dominant gene (bilateral retinoblastoma), or recessive genes (sickle cell anemia).

The aquatic toxicologist needs to know the defined use of the water before assessing the information accumulated thus far. Criteria for industrial use, human drinking water, and farm irrigation may well differ. For example, liquid manure is sprayed onto farm land (acceptable) but is not potable (unacceptable). It is probable that a different battery of tests will be used, dependent upon the defined uses. If one can climb over that hurdle, or is it swim up that fish ladder, then consideration must be given to the relation of that battery of tests to the actual situation. It has been shown that fish, insects and plants consistently accumulated compounds less efficiently from Red River water than from dechlorinated Winnipeg water (Lockhart et al. 1982). Acclimatization occurs with ammonia and chlorine (Lloyd and Orr, 1969). The survival of fish fed five strains of brine shrimp nauplii ranged from 82 to 57 percent (Beck and Bergstrom, 1982). The pH affects the toxicity of ionizable compounds, e.g.,phenol derivatives (Kaila and Saarikoski, 1977). Quantitative structure activity relationships (QSAR), a concept given much impetus by Hansch and co-workers (1971) are a means of identifying the biological-chemical interaction potential (or dose) as opposed to concentration (Ferguson, 1939). Cytochrome P$_1$450 is induced by β-naphthoflavone (Elcombe *et al*. 1979) and arylhydrocarbon hydroxylase by petroleum components (Payne and Penrose, 1975). Oxidation proceeds more slowly than hydrolysis or conjugation. These findings of sorption, acclimatization, nutrition, pH, QSARs, and metabolism are not unique to aquatic toxicology; similar findings are documented in mammalian toxicology. They do indicate some of the difficulties encountered when one tries to extrapolate from laboratory to field. It seems that it is at least as difficult to predict effects on the environment as it is to predict effects on man.

Before estimating the effects on the target species, both toxicologists might conduct some further studies:

a) sub-acute
b) chronic
c) reproduction
d) genetic
e) immunological
f) behavioural
g) neurological

This is proper. But at what point will all this knowledge outstrip our wisdom to understand it?

We should not forget that in order to estimate effects on individuals or populations we must correlate morbidity and mortality with normal biological or pathological changes. Statistically, some of us will have biochemical, structural, or physiological differences that will set us apart from the "normal" human being. It is important to understand whether these differences are, in fact, significant or insignificant either for our own well-being or that of the survival of the species. As you will realize, I use the word "significant" in its biological rather than its statistical sense. In attempting to estimate effects on individuals or populations it may be technically difficult to test the aquatic species of concern and it is certainly legally and morally difficult to test human subjects. Therefore, both the aquatic and the mammalian toxicologist share the problem of extrapolation to other species, for example, to indigenous aquatic species or to man. Really, is it relevant to extrapolate from a rainbow trout to indigenous fish species of Alberta waters; or from rats and pigs to man. Before one can extrapolate, one must show that those metabolic and kinetic parameters discussed earlier are similar in both the animal model and the species of concern.

Genetic toxicology is the microcosm of the problem of extrapolation. Arising out of the current obsession of our society to prevent cancer, we have a large number of short term bioassays to detect mutagenesis and which are used as indicators for carcinogenic potential. Lest any aquatic toxicologist believe that this is only of concern for the human species, let me remind you that mere heterozygosity for a deleterious recessive mutation may incur disadvantages with regard to viability despite the phenotypic dominance of the unmutated gene. In other words, neoplasia are not the only biological end-points which may adversely affect a population. Indeed, it is unfortunate that there are no clear-cut ways to predict genetic effects. Considerable effort is required to establish correlations and to reduce to a minimum the possibilities of obtaining either false positive or false negative results. Mutagenicity testing alone cannot define a compound as a carcinogen. To conduct carcinogenicity testing on whole animals at low ambient levels requires very large numbers of animals.

In this brief presentation I hope that I have persuaded you not to regard aquatic toxicology as something uniquely different from general toxicology. After all, the aquatic environment includes a wide range of species producing zootoxins. Some examples would include:

Protozoa - dinoflagellates
Porifera - sponges
Colenterata - Portugese man-o-war, jellyfish, anemones, coral
Echinodermata - sea urchins
Mollusca - snails, shellfish, octopus
Chordata - stingrays, weever fish, sea snakes, sharks, and puffer fish.

Every year deaths are recorded of human beings who had a close encounter of the terminal kind with Portugese man-o-war, paralytic shellfish poisoning or puffer fish toxicity. On land there is the Russell viper and the African puff adder and, more closely to home, the rattlesnake. Whilst still in the terrestrial environment, let us remember that the duckbill platypus, spiny anteater, and some shrews also produce zootoxins. Although there are obvious differences between the two environments, the zootoxins produced by the respective inhabitants act along well known comparative toxicological principles. May I remind you that the polar bear, bearded seal and walrus induce toxicity when their livers are consumed as human or animal food. The cow and the whale suckle their young through similar physiological mechanisms. Thus, is it not more probable than improbable that xenobiotics, and that mysterious industrial product, will be metabolized through similar pathways of oxidation, reduction, hydrolysis, conjugation, acetylation or thiocyanate formation, whether microsomal or non-microsomal?

In conclusion, aquatic toxicology has accumulated a considerable body of knowledge on the whole-animal responses to xenobiotics. Mammalian toxicology has accumulated a considerable body of knowledge on the anatamo-physiological changes in addition to whole-body responses. In dealing with that industrial product mentioned at the beginning, it doesn't matter whether this is a new antibiotic or industrial effluent. Both will affect eukaryotic and prokaryotic populations, both may cause beneficial or deleterious effects, and both may emerge in the environment. I close with one suggestion, one request and one question. The suggestion is that there be more work on evaluation and prediction of adverse effects in the field of aquatic toxicology. The request is that there be improved communication and dialogue between aquatic and mammalian toxicology. The question is this: Is aquatic toxicology a biological discipline or a multi-disciplinary field?

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AQUATIC TOXICOLOGY - QUO VADIS OR WHERE DO WE GO FROM HERE?

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Aquatic toxicology is constrained by a self image which tends to limit its work to the preservation of aquatic populations and aquatic ecological relationships. A more expansive view would have it concerned with the health and stability of water systems including but not limited to aquatic biology.

The "practice" of aquatic toxicology seems to have plateaued at the LC_{50}. Yet this test (and its equivalents) is not the end point of toxicologic testing but only the beginning. This test does provide an indication of potentially acute toxic effects to aquatic populations in the immediate vicinity. However it tells us relatively little about the general or long term effects on the health of the receiving water system.

Can the techniques and methodologies of mammalian toxicity testing be adapted to aquatic work. The answer is a qualified yes. Unless the toxic principle, the dose, the target organ(s) and the toxic mechanism are known, toxicity testing cannot truly be said to have occurred. It is in answering these questions that aquatic toxicologists can benefit from the approaches in place or being introduced in other branches of toxicology. Finally, toxicology is an integral and inseparable symbiotic relationship between chemistry and biology. Unless aquatic toxicology includes an appropriate measure of each, it will continue to lag somewhat behind the general field of toxicology. Aquatic toxicology is too important to permit that to occur.

Key Words: Aquatic toxicology, water systems, mammalian toxicity.

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La toxicologie aquatique se trouve contrainte par sa propre image qui tend à restreindre ses travaux à la préservation des populations aquatiques et des relations écologiques dans le milieu aquatique. Une perspective plus lareg l'entraînerait à s'intéresser à la salubrité et la stabilité des milieux marins, dont la biologie aquatique, mais sans s'y limiter.

La pratique de la toxicologie aquatique semble s'être arrêtée au CL50. Pourtant, ce test(et ses équivalents) n'est pas le point ultime des analyses toxicologiques mais seulement le début. Ce test fournit une indication des effets toxiques potentiellement graves pour les populations aquatiques dans le milieu immédiat. Il nous révèle relativement peu de choses cependant en ce qui a trait aux effets généraux ou a long terme sur la salubrité du milieu marin.

Les techniques et méthodologies employées dans les tests de toxicologie chez les mammifères peuvent-elles être adaptées au domaine aquatique? Il nous faut répondre d'un oui mitigé à cette question. A moins que l'agent toxique, sa concentration, le ou les organes visés et le mécanisme toxique ne soient connus, on ne peut parler d'analyse de toxicité. C'est en répondant à ces questions que les toxicologues aquatiques pourront tirrer profit des approches déjà existantes ou en voie d'être adoptées dans d'autres branches de la toxicologie. Enfin, la toxicologie est en relation de symbiose étroite entre la chimie et la biologie. A moins d'incorporer suffisamment de ces deux disciplines, la toxicologie aquatique continuera d'être à la remorque des autres domaines de la toxicologie en général. Et elle est trop importante pour laisser une telle chose se produire.

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